DIABETES

The Journal of the American Diabetes Association

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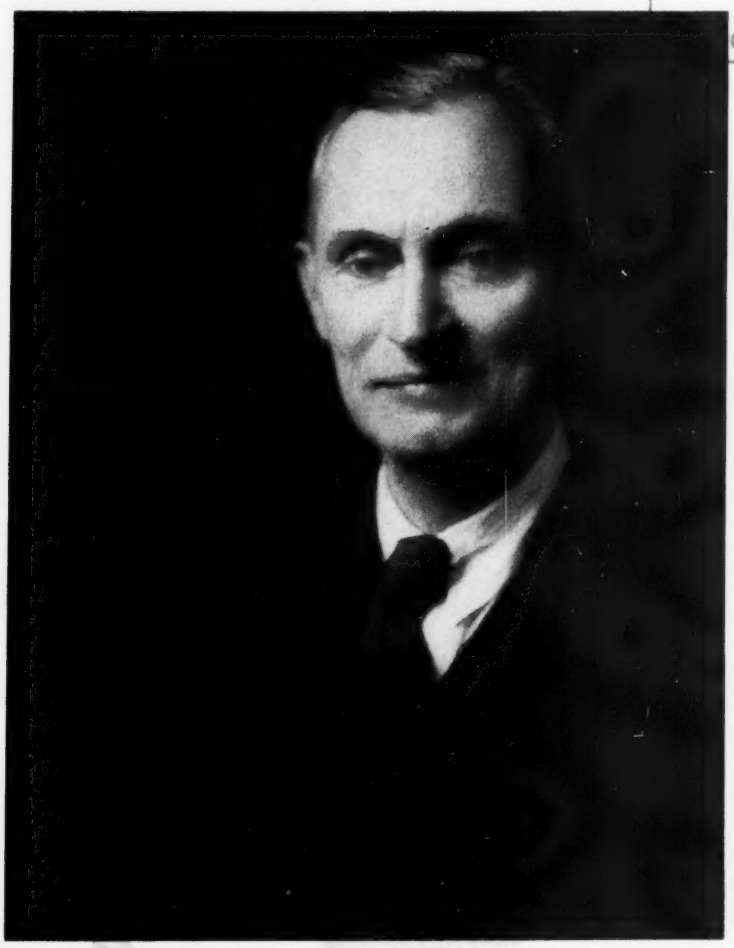

EUGENE L. OPIE

VOLUME 7, NUMBER 6

NOVEMBER-DECEMBER 1958

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Observations Concerning a Humoral Insulin Antagonist During Diabetic Ketosis

James B. Field, M.D., Bethesda*

Two years ago at these meetings we reported on our observations concerning a humoral insulin antagonist in the sera of some patients during diabetic acidosis.[1] Today I should like to review briefly those studies and in addition discuss some of our more recent findings.[2-4]

The technic utilized in most of these studies is based on the fact that insulin augments the glycogen deposition by the isolated rat hemidiaphragm.[2] One hemidiaphragm is exposed to 1 ml. of test serum and 1 ml. of phosphate-saline buffer containing 0.2 units of insulin for one minute, while the control hemidiaphragm is exposed only to 1 ml. of the test serum and 1 ml. of buffer. Subsequently they are incubated in glucose and buffer for ninety minutes, and then the glycogen content of each is determined. The insulin effect is regarded as the extra glycogen synthesized by the hemidiaphragm dipped in insulin as compared to its control. Since both hemidiaphragms were exposed to the same test serum, a significant decrease in the insulin effect is interpreted as evidence for the presence of an insulin antagonist. Table 1 indicates that in the presence of normal sera the insulin effect is 5.35 micromoles of glycogen per gram of diaphragm muscle. In contrast, sera from some patients in diabetic acidosis were found either to abolish or significantly decrease the insulin effect. Several days after the episode of acidosis, insulin antagonist was no longer present (table 1). In two other cases studied, antagonist activity had disappeared from the serum six to ten hours following the institution of therapy for diabetic acidosis.[1] However, in case V and VI no inhibition of the insulin effect during diabetic acidosis was demonstrable (table 1).

Table 2 summarizes the findings in a case of diabetic acidosis who received 21,000 units of insulin during the first twenty-four hours of therapy. Circulating insulin antagonist was still present when as little as 0.01 to 0.05 ml. of serum was used in the test system. It will be recalled that in the previous studies, 1 ml. of serum was used in the assay. The finding of a very high concentration of insulin antagonist is consistent with the clinical picture of extreme insulin resistance during diabetic acidosis.

The clinical data from these seven cases are presented in table 3. There was no correlation between the presence of insulin antagonist and the level of the blood sugar or plasma carbon dioxide content at time of admission in diabetic acidosis. However, there was a general relationship between the amount of insulin that the patient received in the first twenty-four hours and the ability to demonstrate insulin antagonism. None of the patients had exhibited any evidence of insulin resistance prior to the onset of diabetic acidosis, and in the cases who survived, there was no indication of insulin resistance once the acidosis had been treated. Case III was a new diabetic who had never had previous insulin therapy, yet insulin antagonist was demonstrable during diabetic acidosis.

The concomittant increased adrenal cortical activity during diabetic acidosis has been suggested as the cause of the temporary insensitivity to insulin.[5] The addition of large amounts of hydrocortisone to normal serum did not produce insulin inhibition (table 4). Furthermore, two of the cases of diabetic acidosis without demonstrable insulin antagonist had very high levels of circulating hydrocortisone. Serum obtained from a diabetic patient given a forty-eight-hour infusion of adrenocorticotropin (ACTH) to stimulate the adrenals maximally did not antagonize insulin. Table 5 indicates that sera from patients with acromegaly and diabetes did not inhibit the insulin effect in the test system. Maximal adrenal stimulation with ACTH in one of these acromegalics did not produce demonstrable insulin antagonist. The addition of monkey growth hormone singly or in combination with hydrocortisone to normal serum did not result in insulin inhibition (table 6). Serum

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*Winner of the Lilly Award of the American Diabetes Association for 1958.

TABLE 1

Effect of normal and diabetic acidosis serum on the combination of insulin and rat diaphragm

Source of Serum		Amount of Serum cc.	Insulin Effect micromoles (glucose equiv.) per gram of tissue Mean ± S.E.M.	
No serum present		0	6.31 ± 1.71 (3)*	
Normal persons		1	5.35 ± 0.48 (23)	
Diabetic acidosis:			During acidosis	After acidosis
Case I	7/14/54	1	0.75 ± 2.22 (3)	—
	7/17/54	1	—	1.11 ± 0.86 (3)
	7/20/54	1	—	6.60 ± 2.09 (3)
Case II	10/23/54	1	3.11 ± 1.13 (5)	—
	10/25/54	1	—	6.54 ± 0.95 (3)
Case III	11/25/54	1	0.79 ± 1.43 (3)	—
	12/7/54	1	—	6.25 ± 0.32 (3)
Case IV	12/4/54	1	—0.56 ± 1.85 (4)	—
	12/7/54	1	—	6.59 ± 1.02 (3)
Case V	3/16/55	1	5.39 ± 0.63 (3)	—
Case VI	6/11/55	1	6.08 ± 1.11 (3)	—

( )* Number of determinations. (From Field, J. B., and Stetten, D., Jr., *The American Journal of Medicine 21*:340-42, September 1956.)

TABLE 2

Studies on patient in diabetic acidosis receiving 21,000 units of insulin

Source of Serum	Amount of Serum cc.	Insulin Effect micromoles (glucose equiv.) per gram of tissue Mean ± S.E.M.
Case VII		During acidosis
6/14/55	1	—2.62 ± 1.73 (3)*
6/14/55	0.5	—1.44 ± 2.34 (3)
6/14/55	0.2	0.39 ± 0.87 (3)
6/14/55	0.05	1.03 ± 1.32 (3)
6/14/55	0.01	2.89 ± 2.17 (3)
6/14/55	0.5 heated	0.65 ± 0.14 (3)

( )* Number of determinations.
(From Field, J. B., and Stetten, D., Jr., *The American Journal of Medicine 21*:340-42, September 1956.)

from a hypophysectomized human given human growth hormone did not antagonize insulin even though the patient exhibited some evidence of insulin resistance.[6] These studies suggest that the humoral insulin antagonist present in some cases of diabetic acidosis is neither hydrocortisone nor growth hormone. It also seems unlikely that this insulin antagonist is glucagon since the addition of large quantities of glucagon to normal serum did not inhibit the insulin effect.[5] Furthermore, the intravenous injection of insulin antagonist serum into a rabbit caused no significant rise in the blood sugar, while a rapid rise followed the injection of small amounts of glucagon.[5]

The fact that insulin antagonist activity was non-dialyzable but was destroyed by heating to 100° C. for four minutes suggested that it might be a protein.[1] Table 7 presents results obtained from protein fractionation of antagonist serum utilizing starch block electrophoresis. The bulk of the insulin antagonist activity resided in the alpha-1 globulin fraction while the alpha-2 globulins also appeared to have some activity, probably due to contamination with alpha-1 globulins. The albumen, beta and gamma globulins were completely devoid of insulin antagonist. Although the insulin antagonist migrated electrophoretically with the alpha-1 globulins, there was no elevation in the total amount of this protein fraction in several of the cases where insulin antagonist was demonstrable, including case VII, who had a very high concentration of antagonist.[3] The alpha-1 globulins are composed of many different proteins, and the insulin antagonist probably represents only a small per cent of the total fraction. Hence any increase in this antagonist, even a large one, might not produce an elevation in the total alpha-1 globulins. The alpha globulins are rich in lipoproteins but repeated freezing and thawing of antagonist serum did not destroy its activity.[4] In addition, the lipoproteins from antagonist serum were obtained by ultracentrifugation and were found to be devoid of insulin antagonist. The activity was found in the residual serum after the lipoproteins were separated.[4]

In an attempt to learn more about the properties of this insulin antagonist, studies have been done utilizing

TABLE 3

Pertinent clinical data on cases of diabetic acidosis

Case, Sex, Age	Date	Total Insulin on this Date	Blood Sugar (mg. per cent)	CO_2 (mEq./L.)	Remarks
I. F, 29	7/14/54	2,130	560	3	Diabetes for 7 yr.; insulin started 3 wk. prior to episode of acidosis; regulated on 40 units/day; no complications of diabetes
	7/17/54	525	350	27	
	7/20/54	0	52	26	
II. F, 30	10/23/54	615	648	11	Diabetes for 25 yr.; usually regulated on 30-60 units of insulin/day; retinopathy but no other complications
	10/25/54	100	250	28	
III. F, 64	11/25/54	1,310	600	8.3	Diabetes for 5 mo.; no previous insulin; discharged on 80 units NPH/day
	12/7/54	80	264	—	
IV. F, 50	12/4/54	230	200	19	Diabetes for 4 yr.; previously regulated on 25-40 units/day; discharged on 80 units/day; no complications of diabetes
	12/7/54	80	—	—	
V. M, 24	3/16/55	200	346	18	Diabetes for 11 yr.; previously regulated on 120 units/day; discharged on 82 units/day; no complications
VI. F, 45	6/11/55	230	1,025	11	Diabetes for a few weeks; no previous insulin; discharged on 35 units
VII. F, 22	6/14/55	21,000	908	3.5	Diabetes for 8 yr.; previously regulated on 60 units/day; no complications of diabetes; peritonitis found at autopsy

(From Field, J. B., and Stetten, D., Jr., *The American Journal of Medicine 21*:340-42, September 1956.)

TABLE 4

Role of adrenal cortical hormones in the insulin effect on rat diaphragm

Source of Serum	Plasma Level of Compound F* (μg. per cent)	Insulin Effect micromoles (glucose equiv.) per gram of tissue Mean ± S.E.M.
Normal serum to which was added 100 μg. per cent compound F	>100	6.45 ± 1.25 (3)†
Case V	58.2	5.39 ± 0.63 (3)
Case VI	102	6.08 ± 1.11 (3)
Diabetic patient at end of 48-hr. infusion of 200 units ACTH	40	5.18 ± 1.23 (6)
Normal serum	—	5.35 ± 0.48 (23)

* Normal range: 8-24 μg. per cent.
( )† Number of determinations.
(From Field, J. B., and Stetten, D., Jr., *The American Journal of Medicine 21*:340-42, September 1956.)

digestive enzymes. Table 8 indicates that treatment of the antagonist serum with trypsin does not destroy its activity. On the other hand, table 9 demonstrates the inactivation of the inhibitor after chymotrypsin digestion. Following the chymotrypsin treatment of the serum and before it was assayed for antagonist, it was necessary to add di-isopropylfluorophosphate, a chymotrypsin inhibitor, to prevent the insulin in the test system from also being destroyed. Di-isopropylfluorophosphate, by itself, had no effect on the activity of the insulin antagonist. These observations are consistent with the notion that the insulin antagonist is a protein but that it does not contain arginine or lysine in a position crucial to its activity.

The insulin inhibitor was still active whether the rat diaphragm was dipped in insulin before or after it had been exposed to the antagonist serum (table 10). This suggests that the insulin antagonist is attached to the rat diaphragm independent of the insulin and does not act by interfering with the binding of insulin to muscle. After both the insulin and antagonist have combined with muscle there is some kind of interaction between them which produces inhibition of the insulin effect. In this respect, the insulin antagonist which is present

TABLE 5

Inability of serum from diabetic acromegalics to inhibit insulin effect

Source of Serum	Amount of Serum ml.	Insulin Effect micromoles (glucose equiv.) per gram of tissue Mean ± S.E.M.
H.R. 52 yr. female	1	5.93 ± 0.95 (6)*
A.L. 55 yr. female	1	5.24 ± 0.64 (6)
A.L. — at end of 48 hr. infusion of 200 units ACTH	1	5.41 ± 1.27 (6)

( )* Number of determinations.
(From Field, J. B., and Stetten, D., Jr., DIABETES *5*: 391-96, 1956.)

TABLE 6

Failure of growth hormone to inhibit insulin effect

Material tested	Amount ml.	Insulin Effect micromoles (glucose equiv.) per gram of tissue Mean ± S.E.M.
Monkey Growth Hormone 160 γ/ml.	1	6.49 ± 1.60 (3)*
Monkey Growth Hormone 160 γ/ml. + Hydrocortisone 4 γ/ml.	1	7.70 ± 2.10 (3)
Serum from patient given human growth hormone	1	8.37 ± 1.41 (3)

( )* Number of determinations.
(From Field, J. B., Tietze, F., and Stetten, D., Jr., *The Journal of Clinical Investigation 36*:1588, 1957.)

TABLE 7

Activity of various protein fractions of insulin antagonist serum

Fraction	Equivalent Amount of Whole Serum ml.	Insulin Effect micromoles (glucose equiv.) per gram of tissue Mean ± S.E.M.
Albumin	0.15	7.23 ± 0.74 (3)*
α_1-Globulins	0.15	1.41 ± 0.99 (3)
α_2-Globulins	0.15	4.72 ± 2.26 (3)
β-Globulins	0.15	8.52 ± 1.77 (3)
γ-Globulins	0.15	8.17 ± 2.64 (3)

( )* Number of determinations.
(From Field, J. B., Tietze, F., and Stetten, D., Jr., *The Journal of Clinical Investigation 36*:1588, 1957.)

TABLE 8

Failure of trypsin digestion to inactivate serum insulin antagonist

Treatment of Serum	Amount of Serum ml.	Insulin Effect micromoles (glucose equiv.) per gram of tissue Mean ± S.E.M.
Trypsin Digested Serum Case IX	1	—0.03 ± 0.84 (3)*
Untreated Serum Case IX	1	1.40 ± 0.43 (8)
Trypsin Digested Normal Serum	1	4.28 ± 0.85 (12)

( )* Number of determinations.
(From Field, J. B., Tietze, F., and Stetten, D., Jr., *The Journal of Clinical Investigation 36*:1588, 1957.)

TABLE 9

Inactivation of insulin antagonist by chymotrypsin digestion

Treatment of Serum	Amount of Serum ml.	Insulin Effect micromoles (glucose equiv.) glycogen per gram of tissue Mean ± S.E.M.
Untreated Serum Case IX	1	1.40 ± 0.43 (8)*
Chymotrypsin + DFP + Serum Case IX	1	7.79 ± 1.98 (3)
Untreated Serum Case VII	.05	1.03 ± 1.32 (3)
DFP + Serum Case VII	.05	1.13 ± 1.68 (3)

( )* Number of determinations.
(From Field, J. B., Tietze, F., and Stetten, D., Jr., *The Journal of Clinical Investigation 36*:1588, 1957.)

TABLE 10

Ability of insulin antagonist serum to abolish insulin effect when diaphragm is exposed to it and insulin separately

Procedure	Amount of Serum ml.	Insulin Effect micromoles (glucose equiv.) per gram of tissue Mean ± S.E.M.
Pre-exposure of diaphragm to serum	0.2	0.71 ± 2.04 (3)*
Pre-exposure of diaphragm to insulin	0.2	1.15 ± 0.46 (3)

( )* Number of determinations.
(From Field, J. B., and Stetten, D., Jr., DIABETES *5*: 391-96, 1956.)

TABLE 11

Failure of serum from insulin resistant diabetic acidosis patient to inhibit binding of I^{131}-insulin to rat diaphragm

Source of Serum	Number of Experiments	Counts/min. of I^{131}- bound/gm. of diaphragm using I^{131}-insulin ± S.E.M.
Normal	6	2,683 ± 204
Case VII	6	2,758 ± 144

(From Field, J. B., and Stetten, D., Jr., DIABETES 5: 391-96, 1956.)

during diabetic acidosis differs from the circulating insulin inhibitor which can be demonstrated in some cases of chronic insulin resistance. When these same kinds of experiments are done using serum from patients with chronic insulin resistance, antagonism can only be demonstrated when the serum, insulin and diaphragm are all in contact at the same time.[7,8] Serum from chronic insulin resistant patients no longer has any antagonist activity when the rat hemidiaphragm is exposed to it first, then washed and subsequently dipped in insulin. Table 11 further supports the idea that the insulin antagonist present during diabetic acidosis did not interfere with the binding of insulin by rat hemidiaphragm. Utilizing I^{131}-labeled insulin there did not appear to be any difference in the amount of insulin bound to rat hemidiaphragm in the presence of insulin antagonist or normal serum. In other studies with insulin I^{131} no "insulinase" activity in the insulin antagonist serum was demonstrable.[3] It thus appears unlikely that this insulin antagonist acts by increasing insulin destruction.

Since beef or pork insulin had been used in all these experiments it was natural to wonder whether the insulin antagonist would also inhibit human insulin. Table 12 summarizes experiments done with insulin prepared from human pancreas by Dr. Frank Tietze of the National Institute of Health.[3] The insulin antagonist also abolished the effect of human insulin in the rat diaphragm system. This observation coupled with the fact that we have found antagonist in the serum of a new, previously untreated diabetic patient in acidosis raises the possibility that this factor might be of importance in the etiology of some cases of human diabetes.

It should be emphasized again that all these studies were done using 0.2 units of insulin in the test system. This concentration of insulin is perhaps 1,000 times the normal physiologic concentration.[9] In order to demonstrate inhibition of this much insulin, it is obvious that the serum must contain a high concentration of antagonist, and this might explain our failure to demonstrate

TABLE 12

Inhibition of human insulin by insulin antagonist during diabetic acidosis

Serum	Type of Insulin		Insulin Effect micromoles (glucose equiv.) glycogen per gram of tissue Mean ± S.E.M.
Diabetic Acidosis	Beef-Pork	0.2 unit/ml.	1.32 ± 2.02 (3)*
Diabetic Acidosis	Human	0.2 unit/ml.	—0.30 ± 1.58 (3)
Normal	Human	0.2 unit/ml.	6.48 ± 0.06 (3)

( )* Number of determinations.
(From Field, J. B., Tietze, F., and Stetten, D., Jr., *The Journal of Clinical Investigation* 36:1588, 1957.)

its presence in all cases of diabetic acidosis. During the past year we have continued this work at the Diabetic Unit of King's College Hospital in London in collaboration with Mrs. Barbara Rigby of the Department of Chemical Pathology.[4] We have modified our test system so that instead of 0.2 units of insulin we now use only 0.01 units. With this more sensitive assay we have been able to demonstrate a circulating insulin antagonist in the sera of several new diabetic patients before they received insulin therapy.[4] This observation confirms the previous findings of Vallance-Owen.[10] Although these patients had glycosuria and ketonuria, they were certainly not in diabetic acidosis. Several days after the institution of insulin therapy and at a time when the diabetes was controlled, insulin antagonist was no longer demonstrable. This is very similar to the behavior of the insulin antagonist during diabetic acidosis. In addition, using this more sensitive test system we have also found antagonist in the sera of several patients whose diabetes was aggravated by an acute infection.[4] It is too early to know whether the antagonist which we have found in the new diabetic patients and the diabetics with an infection is the same entity as that which we have found in diabetic acidosis. It is hoped that studies now in progress will answer this question.

SUMMARY

We have been able to detect a circulating insulin antagonist in the sera of some patients in diabetic acidosis. This antagonist behaves like a protein since it is nondialyzable, inactivated by heating to 100° C. for four minutes, and migrates electrophoretically with the alpha-1 globulins. It is not a lipoprotein and is destroyed by chymotrypsin digestion. It does not appear to be either hydrocortisone, growth hormone, glucagon or insulinase.

It does not interfere with the binding of insulin to muscle but seems to exert its inhibitory effect after both it and insulin have become attached to muscle. It is also capable of antagonizing insulin prepared from human pancreas.

SUMMARIO IN INTERLINGUA

Observationes Relative A Un Antagonista Humoral A Insulina, Occurrente Durante Cetosis Diabetic

Nos ha detegite un circulante antagonista a insulina in le seros de certe patientes in stato de acidosis diabetic. Le antagonista se comporta como un proteina, in tanto que illo es non-dialysabile, que illo es inactivate per calefaction a 100 C durante quatro minutas, e que illo migra electrophoreticamente con le globulinas alpha-1. Illo non es un lipoproteina e illo es destruite per digestion a chymotrypsina. Apparentemente illo es ni hydrocortisona, ni hormon de crescentia, ni glucagon, ni insulinase. Illo non obstrue le ligation de insulina a musculo sed pare exercer su effecto inhibitori post que illo mesme e le insulina es attachate a musculo. Illo ha etiam le capacitate de antagonisar insulina preparate ab pancreas human.

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Experimental Xanthomatosis

The feeding of cholesterol to rabbits for prolonged periods results in the deposition of cholesterol in the skin, joints and bones, and produces lesions closely resembling hypercholesterolemic xanthomatosis in man. Several aspects of this disease have been reviewed recently (*Nutrition Reviews 11*:72, 231, 264, 1953).

C. I. Wang, L. Strauss and D. Adlersberg (*Arch. Path.* 63:416, 1957) reported their study of the characteristics of experimental xanthomatosis in the rabbit including the incidence, clinical and roentgenological manifestations, blood lipid changes and gross and microscopic changes in the skin, joints and bones. The animals used in this experiment consisted of a group of 120 male rabbits weighing between 2.5 and 3.0 kg. which received about 1 gm. of cholesterol daily from their diet of cholesterol-treated chow. The feeding intervals ranged from two weeks to one year. Blood samples taken every two to four weeks were used to determine the amounts of circulating cholesterol, phospholipids and total lipids.

The development of xanthomata in the skin required hypercholesteremia, but there was no correlation between the degree of elevation of blood cholesterol and the time of appearance of the skin xanthomatosis. The duration of cholesterol feeding was an influential factor. The variation in lipid deposition between individuals could have been due to local tissue factors varying from animal to animal.

No gross atheromatous plaques were seen until the end of one month of feeding. Tiny, but definite, white plaques could be seen in the ascending part and the arch of the aorta especially around the orifices of the branching arteries. As cholesterol feeding continued, the plaques became larger, more numerous and confluent. Plaques appeared at the orifices of the intercostal arteries and the branching arteries of the abdominal aorta. The mitral and aortic valves became thickened and beaded. Continuation of cholesterol feeding beyond four to six months resulted in such extensive plaque formation in the aorta and pulmonary artery that normal intima could be seen in only occasional small areas.

From *Nutrition Reviews*, Volume 16, Number 7, 1958, pp. 211-12.

Management of the Pregnant Diabetic

Walter S. Jones, M.D., Providence

INTRODUCTION

Among the 140,665 deliveries at the Providence Lying-In Hospital in the thirty-one year period 1927-1957, 169 diabetic women were observed in 273 pregnancies. Weeding out abortions, dubious diagnoses and cases delivered elsewhere, there remain 251 viable pregnancies on which this study is based.

Although this is the fifth largest obstetrical service in the country, the mechanics of organization and physician-patient relationship are comparable to any large community hospital not directly connected with a teaching center. In the management of the pregnant diabetic, we experience certain disadvantages common to all such institutions:

1. Local industrialization and the prevalence of prepaid insurance coverage have created a progressive shift from clinic to private patient status. About seven eighths of our current clientele is private. This is a closed-staff hospital, but because of its size the staff is large. Consequently in the last ten-year period, only about one fifth of the diabetics were the clinic's responsibility. The ninety-three private deliveries were conducted by twenty-six different obstetricians, and forty-three internists and general practitioners participated in their medical management. This *lack of uniformity of control* is in marked contrast to the teaching hospital, where a select group personally supervises most of the diabetics.

2. Limitation of funds induces patient resistance to the long periods of hospitalization desirable in the last trimester.

3. Most important to remember, the community obstetrical service is obliged to accept emergency cases and miscellaneous drifters, infrequently seen in specialized centers. This has a devastating effect on fetal loss statistics.

Our problems are those of the majority of hospitals, but size permits accumulation of a large body of material in a shorter time. A review of our experience might be informative, in seeking the areas where results may be improved.

In light of modern knowledge, it may seem primitive to revert to discussion of such fundamentals as ketosis and toxemia. Yet these continue to be major causes of fetal death. A refreshingly candid appraisal from the Johns Hopkins Hospital[1] showed that one half of the fetal loss occurred in utero, three quarters of it in women under poor diabetic control; and 18.8 per cent of the total fetal loss was associated with significant degrees of toxemia. Among 119 diabetics in a Belfast, Ireland, clinic[2] there were seven instances of coma, with 71.4 per cent loss, and nineteen cases of pre-eclampsia, with 25.2 per cent wastage. This could be expanded into a large bibliography of error in simple medical care, by no means always the responsibility of the obstetrician, and often the fault of the patient herself.

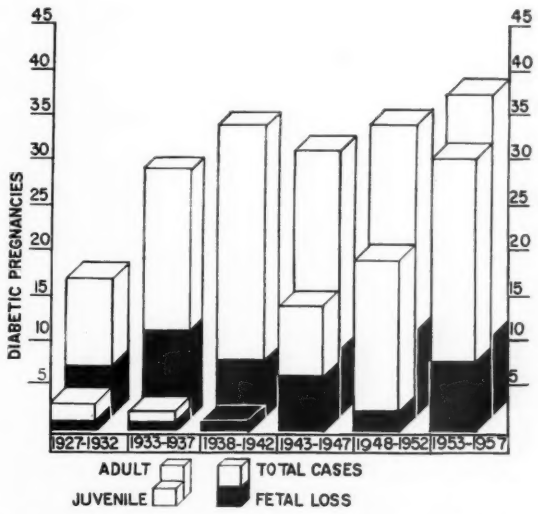

FIG. 1. Five-year periods—type of patients and fetal loss.

DEFINITION OF TERMS

In this study no distinction is made between "old" and "new" series, nor between clinic and private patients.

Unless otherwise specified, the entire viable fetal loss among diabetic patients during thirty-one years is counted. This includes intra-uterine death known to have occurred before acceptance of the case. It also includes infants leaving this hospital alive to die elsewhere, within two weeks, of congenital anomaly.

From the Providence Lying-In Hospital, Providence, Rhode Island.

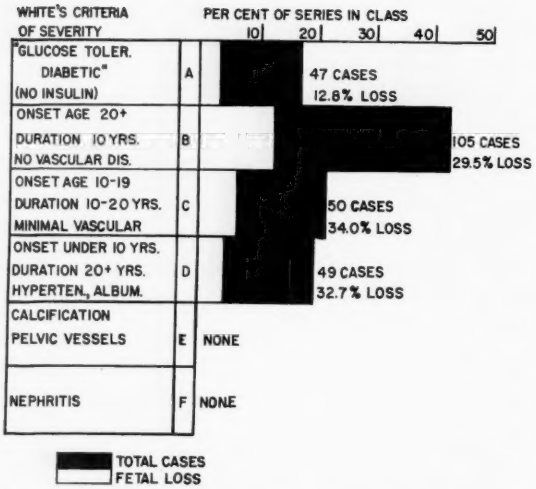

FIG. 2. Distribution and fetal loss by White's classification.

White's original criterion[3] of 960 gm. (2 lb., 2 oz.) for viability is still used, although we[4] regard this as an unrealistically low level. If, as in some reports, the standard is raised to 1,500 gm. (3 lb., 5 oz.),[5] or to about twenty-eight weeks,[6,7] the basic fetal survival here recorded would improve by 5 per cent across the board.

White's classification of severity[3,8] is followed for uniformity, although we do not concur in all its details.[9] The salient features of age of onset and duration of disease involved in this scheme are indicated in figure 2.

"Juvenile" denotes a diabetic diagnosed before age twenty; "adult" or "older women" acquired the disease afterwards. This is the basic metabolic background of the individual. The status at any given pregnancy is further clarified by specifying a disease span of "less than ten years" or of "ten-year" duration.

There is no desire to make the fetal salvage look good. This unvarnished account of rather mediocre results seeks the answer to two questions: What kind of diabetic women lose their babies; and under what circumstances? Statistics are a tool to pin-point trouble spots where corrective measures may be intelligently applied.

POTENTIALLY DANGEROUS CASES

The profession has been thoroughly alerted to the hazards of pregnancy in the juvenile diabetic. With attention focused in that direction, there is a tendency to overlook certain other areas of fetal loss.

1. *Fetal loss in older women.* There is a rising incidence of juvenile cases by five-year periods (figure 1). Despite the increasing number of these young women, there is a striking improvement in their fetal salvage. The perinatal loss in juvenile diabetes in the last ten years is less than half that of the preceding period. On the other hand, although the proportion of adult cases is diminishing, their fetal loss remains constant at about 25 per cent.

2. *Loss in mild diabetes.* When severity is graded by White's classification (figure 2), the fetal loss is 13 per cent in Class A and 30 per cent in Class B. This is a tragic waste in these relatively mild diabetics. Too much of it results from failure to establish the diagnosis until the patient is in serious condition, or from regarding the known mild diabetic too lightly. There is increasing emphasis in the recent literature[10-13] on prompt detection and active management of diabetes unmasked by the current pregnancy.

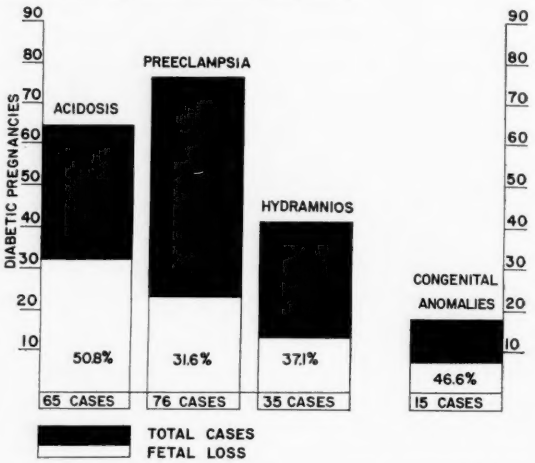

FIG. 3. "The Killers"—complications associated with a high fetal loss.

3. *The role of age and multiparity.* Age and parity influence both the course of the diabetic process itself and the general outcome of pregnancy in these women.

On the first of these points the pattern is familiar: progressively large infants, transient glycosuria, then an abnormal glucose tolerance, and finally frank diabetes. This sequence needs no elaboration.

The role of age and parity in the over-all outcome of the diabetic pregnancy itself is more difficult to evaluate. Results must be assessed on fetal loss; and this picture is obscured by such extraneous complications as toxemia, congenital anomaly, and obstetrical accidents. These are common to all diabetics; they are frequently compounded; and over most of them we have little or no control.

The one complication over which we can exert some effective restraint is acidosis. The types of break in control comprising categories 1-3, figure 4, are considered to be "preventable." If this preventable acidosis could be eliminated, the remaining fetal loss would represent the assorted hazards that beset all diabetics, regardless of age or duration. In the following discussion deaths attributable to "preventable" acidosis have been deleted from the "corrected" computations. When this residual fetal wastage is examined on the basis of age of onset and longevity of disease, some interesting data are elicited:

FIGURE 4

Acidosis—causes and fetal loss

Cause of break in control	Cases	Per cent all acidosis	Fetal loss No.	Fetal loss Per cent
1. Unco-operative patient	21	32.3	13	61.9
2. Inadequate medical supervision	11	16.9	8	72.7
3. Undiagnosed until in acidosis	5	7.7	3	60.0
4. Intercurrent infection	18	27.7	5	27.7
5. "Brittle" diabetic under competent medical management	10	15.4	4	40.0
Total acidosis	65	100.0	33	50.8

The preventable acidosis in categories 1, 2, and 3 represents 56.9 per cent of all acidosis, and is associated with 34.3 per cent of the gross fetal loss in diabetes.

1. *Age versus duration.* The corrected fetal loss in juvenile diabetes is 10 per cent in girls of less than ten years' duration, and goes to 17 per cent in ten-year cases. It is 17 per cent in adult diabetics with short-term disease, rising to 50 per cent in the ten-year patients. In other words, (a) there is a significant increase in fetal wastage in long-standing diabetes; and (b) this is most striking in the older age group of patients.

2. *Variations within the same class of severity.* The same question can be approached from another direction, by comparing the performance of the different types of women who fall by definition into the same severity classification. White's heterogeneous Class D is a good example, since it includes three groups with widely divergent age and metabolic characteristics:

a. First are the dramatic juveniles, all of more than ten years' duration, and most with a high insulin requirement. Their average age was 26.5, and only six were thirty years or older. The gross fetal loss was 16.6 per cent, corrected to 8.3 per cent.

b. Next come the older women with essential hypertension, only two of whom were ten-year diabetics. The average age of those who required insulin was 36.8 years. The age of mothers with dead infants was roughly equal to those whose offspring survived. The gross and the corrected fetal loss here was *more than three times as great* as that of the juveniles in the same class.

c. Last is the handful of essential hypertensives with diabetes so mild as to require no insulin. Their average age was also 36.8. It may be only a sampling error that their gross and corrected fetal loss was *greater* than that of their insulin-treated contemporaries.

This is tenuous evidence, based on a small series of cases. It does not correlate with the concept of severity postulated by White, who places the weight of emphasis on the juvenile diabetics. Nevertheless, the inference is that although parity, duration of disease, and level of insulin demand all sway the result, advancing maternal age is perhaps the determining factor in the over-all outcome of pregnancy in the diabetic.

There is no desire to minimize the serious problems presented by the juvenile patient. We simply emphasize that appreciable fetal salvage may be achieved by more meticulous attention to the older women, many of them mild diabetics of short duration and with low insulin requirement, and some of them even undiagnosed at the outset of pregnancy.

MAJOR COMPLICATIONS

Three clinically detectable physiological complications are associated with the great majority of fetal deaths (figure 3). They may not be the immediate cause of exitus, but they play an important part in the chain of events leading to the intra-uterine death, or to the onset of labor or the manner of delivery resulting in neonatal demise.

1. In the presence of acidosis the fetal loss is 51 per cent.

2. Among all cases of pre-eclampsia 32 per cent of the infants died.

3. Where hydramnios was recorded the wastage was 37 per cent.

4. Congenital anomaly must be mentioned here, although it is seldom detected prior to birth, because it has been so publicized. Our incidence of malformations was three times that of the general population; but this was still a small number, and only half were incompatible with life. Congenital anomaly was not responsible for an important segment of the fetal loss.

ACIDOSIS

Significant ketoacidosis occurred in one quarter of our cases, with deplorable results (figure 4). "Preventable" breaks in control due to unco-operative patients, inadequate medical supervision, and undiagnosed diabetes comprised 57 per cent of all acidosis encountered, and accounted for one third of the gross fetal loss. We are not alone in this regard. Pedowitz and Shlevin[14] report 28.7 per cent acidosis, contributing to two thirds of the perinatal loss, which could "be explained in large measure by poor patient co-operation." This peculiar negativism is characteristic of the emotional make-up of many diabetics.

As preventable acidosis is reduced, the importance of intercurrent infection comes to the fore. Commonest pitfalls are gastrointestinal upsets, urinary infections, and respiratory conditions, in that order. Five of the seven episodes of coma seen in six patients were from these causes. Each woman must be impressed with the danger of even the simplest sources of temperature and fluid loss.

The time of appearance and the lethal effect of acidosis are quite uniform. There were three instances in the nonviable pregnancies, and acidosis was undoubtedly the reason for fetal death in two late abortions. Half of the severe ketosis and two thirds of the coma occurred before thirty-six weeks. The fetal wastage was about 50 per cent both before and after this time point.

This problem points up the flaw in division of responsibility between internist and obstetrician. These crises often arise when the medical member of the team is unavailable. The obstetrician who accepts a diabetic must have a sound working knowledge of the management of precoma.

The other side of the metabolic coin should be mentioned. Really severe insulin reactions occurred only three times, all before thirty-six weeks. One infant succumbed at the time; of the two who survived the immediate insult, one died weeks later of other causes.

TOXEMIA

Pre-eclampsia is the next most important complication, appearing in one third of the series (figure 5). The fetal loss was 22 per cent in mild toxemia, and double that in the severe type. One third of all hypertensives developed superimposed pre-eclampsia, with a 54 per cent wastage. These figures do not include twenty-one cases of otherwise uncomplicated essential hypertension, a not innocuous condition in which 24 per cent of the infants died. There was one case of eclampsia, with fetal survival.

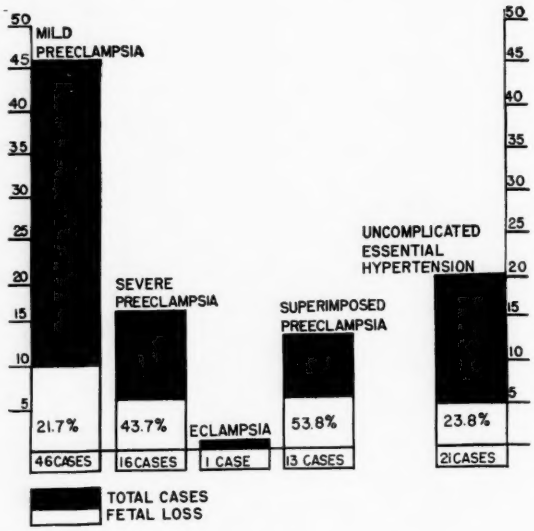

FIG. 5. Toxemia—types and fetal loss.

Management of toxemia is much more difficult than in the normal woman. The diabetic has an inherent tendency to accumulate fluid in all possible bodily water storage spaces, including the uterus. To this little-understood phenomenon is ascribed an endocrine etiology. That this edema is a precursor to pre-eclampsia is evidenced by the consistently high toxemia rate among the diabetics of clinics with excellent results in their other patients.

Water imbalance has been combated without notable success in several ways: (1) Traditional regimes of fluid and sodium ion restriction. (2) Employment of various diuretics, including the sugar and magnesium concentrates, ammonium chloride, mercurial agents, and anhydrase inhibitors. (3) New preparations combining hypotensive and diuretic properties have some promise. (4) One argument advanced for sex hormone therapy is that the method reduces the incidence of toxemia. (5) Drainage of hydramnios has been performed in some clinics.

Instead of the usual porky, edematous, diabetic infant, the products of toxic mothers, especially in essential hypertension, may be underweight. Their fragility is in rough proportion to the period of exposure to the pre-eclampsia. A short attempt at stabilization may be desirable, but it is unwise to temporize with toxemia. Even though the pressure improves, continued intra-uterine existence jeopardizes the fetus.

HYDRAMNIOS

Knowledge of the causes and physiology of hydramnios is meager. It probably occurs to some degree in all diabetics, but is hard to measure quantitatively. As previously suggested, it accompanies profound metabolic disturbance more frequently than it does congenital malformation. Further, there is more than an accidental relationship between the appearance of hydramnios and the development of fetal edema leading to hydrops. Gross distention may be considered a valid indication to terminate pregnancy.

Decompression of the uterus by tapping is still in the experimental stage. The rationale of merely reducing intra-uterine tension in hope of prolonging pregnancy is debatable. The point is what happens to the fetus, whose exposure to the abnormal chemical and physiological environment producing the hydramnios is thus artificially prolonged. Final evaluation will depend on the experience with these infants.

PHILOSOPHY OF MANAGEMENT

The closest internist-obstetrician integration is essential. Our ideas on management are crystallizing along the following lines:

1. *Diabetic control.* All observers agree that *control of the diabetes is of prime importance.* The internist is responsible for prevention of acidosis. Just how rigidly to keep the patient sugar-free is a controversial subject not germane to this review.

2. *Prevention of toxemia.* During the second and early third trimesters the principal function of the obstetrician is to avert toxemia. The final answer to this formidable assignment could be an effective double-acting drug: diuretic to control edema and minimize pre-eclampsia; hypotensive to reduce essential arteriolar spasm, which in turn would diminish superimposed pre-eclampsia.

3. *Female sex hormones.* Hormone therapy is alleged to confer a number of benefits. About 10 per cent of our cases were so treated. One third of these were relatively mild diabetics, the rest severe by any standard. The results did not encourage continuation of the method. In all, twenty-four women received hormones and 227 did not. The only significant difference was a section rate three times as high in the hormone group, because early termination was part of the regime in vogue. The incidence of acidosis and of toxemia, and the fetal loss, were virtually the same in both groups.

4. *Time of delivery.* Approaching term, the big question is how much longer the pregnancy may safely continue. Much has been said about late intra-uterine death, implying a mysterious phenomenon which strikes without warning in the last month. It has been attributed to hormonal imbalance, placental senescence, and other hypothetical causes, none of them adequately verified. The "interventionist" school recommends early termination to forestall this disaster. This seems to offer an easy solution to a difficult problem, but the entire concept is open to doubt.

a. The so-called "senescent placenta" of diabetes is not an established entity, either by clinical observation or by pathological study.

b. Death in utero occurs infrequently without some demonstrable metabolic, anatomical, or obstetrical reason. Only seven of our forty-eight stillbirths could be classified as "unexplained."

c. *Mandatory early delivery.* The thirty-sixth week is repeatedly designated the critical point at which diabetic pregnancies must end, because thereafter things rapidly go wrong. This theory does not seem to stand the test of practice. Interference so early only too often converts a small but living fetus into a premature neonatal death statistic.

Clayton[15] reports 201 cases from London. Termination was usually at thirty-six weeks, involving a section rate of 67 per cent. The fetal mortality was 28 per cent, including twenty-one stillbirths and thirty neonatal deaths. Autopsy showed atelectasis or hyaline membrane disease in 76.6 per cent of the latter.

Louw and Sinclair[16] in Cape Town, South Africa, regard it "a reasonable procedure to interrupt pregnancy soon after the thirty-sixth week." Their perinatal loss in ninety booked cases was seven stillbirths and seven neonatal deaths.

White's group is the best-known American exponent of early delivery. The Boston hospital accommodating most of its patients segregates the statistics in a "Joslin Clinic" column. The last annual report[17] lists seventy-seven infants in seventy-five deliveries. Subtracting nine abortions, the section rate in sixty-six viable pregnancies was 56 per cent. The fruit of this effort was eight stillbirths and thirteen neonatal deaths, a viable fetal loss of 30.9 per cent.

All this is epitomized by Waters' crisp remark[18] that "the fetal loss due to bad timing is just about as high as that from deferred interference." It inspires little confidence in the philosophy that the tribulations of diabetic pregnancy are conveniently solved by early delivery. There is a discernible trend for shrewd observers[19–20] to edge up closer towards term.

We can not subscribe to the belief that intra-uterine death, of itself, takes a sharp upswing after thirty-six

FIGURE 6

Causes of fetal death

Principal cause	Under thirty-six weeks			Thirty-six weeks and over			Total	
	Stillbirth	Neonatal	Total	Stillbirth	Neonatal	Total	No.	Per cent
Acidosis major cause	10	2	12	9	—	9	21	30.0
Toxemia major cause	4	2	6	7	1	8	14	20.0
Congenital anomaly	2	1	3	2	2	4	7	10.0
Prolapsed cord, cord around neck, etc.	3	—	3	1	—	1	4	5.7
Traumatic vaginal delivery	—	—	—	3	—	3	3	4.3
Prematurity (under 2,250 gm. or 5 lb.)	—	8*	8	—	—	—	8	11.4
Atelectasis, hyaline membr., pulm. edema, etc.	—	2	2	—	4	4	6	8.6
"Unexplained" intra-uterine death	4	—	4	3	—	3	7	10.0
Total	23	15	38	25	7	32	70	100.0

*One pair of premature twins counted as a unit.

weeks. Most stillbirths are only secondary reflections of crises in maternal physiology. There were more fetal deaths in our series before thirty-six weeks than after. If the wastage in very small infants produced by spontaneous premature labor is excluded, the loss from all other causes was almost exactly the same in the two groups. With this correction, the incidence of intra-uterine death was 76.7 per cent before and 78.1 per cent from thirty-six weeks on; and the principal reason for these deaths was acidosis and/or toxemia in 60.9 and 64.0 per cent respectively (figure 6).

This bolsters our conviction that there is no happy target date at which pregnancy must automatically be terminated. Although these infants may be handicapped by the maternal metabolic environment and compromised by their own edema, they are entitled to as much maturity as can be obtained for them. Little is gained by interference *unless and until* there arise such complications as instability of control, pre-eclampsia, or hydramnios. If and when this happens, prompt action is in order.

5. *Method of delivery.* The need for intervention brings the final decision of what method to employ. This should depend on the usual considerations of parity, cervical preparedness, and the size and position of the fetus. Of equal importance is evaluation of the ability of mother and infant to withstand a period of stress and trauma. In many instances, including most breech presentations, abdominal delivery will be indicated. Also a policy of carrying as close to term as practicable will result in more large babies, with a corresponding increase of cesareans for disproportion.

We have no antipathy to increasing the section rate in areas that prove constructive. Our current incidence is about 25 per cent, and it will rise as more juvenile diabetics and their repeat operations collect. Published reports, however, are not convincing that rates in excess of 65 per cent can be justified by the salvage achieved.

Nor must we forget that when diabetes itself is the indication for primary section, the girl is condemned to surgery for the rest of her reproductive career. We had one multipara and two primiparas go into spontaneous labor while awaiting section for very acceptable indications; one primipara has had a subsequent normal vaginal delivery; all the infants are alive. This is a saving of four planned operations which proved in retrospect to be unnecessary.

CONCLUSION

Headway in improving fetal salvage in diabetes seems painfully slow. The loss over a thirty-one year span was 28 per cent, and remains close to 25 per cent in recent periods. Much of this loss occurs among the older women and milder cases who should do better. Too much of it stems from small but costly slips in diabetic control and from debatable obstetrical judgment. We believe that improved fetal salvage will come about, not by emptying the uterus earlier or by doing more cesareans,

but largely as the result of more meticulous attention to control of diabetes and a more effective attack on toxemia.

SUMMARIO IN INTERLINGUA

Regime De Gravidas Diabetic

Progresso in salvar vitas fetal in casos de diabete es penosemente lente. Pro un periodo de trenta-un annos, le perdita amontava a 28 pro cento, e pro periodos recente ille valor remane proxime a 25 pro cento. Un grande parte de iste perdita interessa feminas de etates plus avantiate e con diabete comparativemente pauco sever. Isto non deberea esser. Le causa es troppo frequentemente un micre (sed costose) error in le control del diabete o un dubitose judicamento obstetric. Nos opina que progresso in salvar vitas fetal va esser effectuate non per vacuar le utero plus precocemente o per interprender un plus grande numero de sectiones cesaree sed primarimente in consequentia de un plus meticulose attention al detalios del control de diabete e de un plus efficace attacco super le menacia toxemic.

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The first clinical and experimental observations that formed the background for the utilization of certain sulfonamide derivatives in the treatment of diabetes were made during the first half of 1942. At that time, M. Janbon and his co-workers of the Infectious Disease Clinic at the Medical School of Montpellier, were investigating the therapeutic effects of the isopropylthiodiazole derivative of sulfanilamide (2254 R.P.) in typhoid fever. They found that this drug produced in some patients, especially those who were undernourished, symptoms and signs resembling hypoglycemia. Some patients had great neurological disturbances, and died. The chemical data revealed low values for blood sugar. The intravenous injection of glucose tended to alleviate the symptoms in some individuals, but in others it was not helpful. Janbon presented this problem to us and asked for our advice.

Some months earlier we had completed an experimental study with Hedon and Heymann of the irreversible neurological lesions produced in the dog by large doses of Protamine Zinc insulin. It occurred to me that there might be a parallelism between the effects of 2254 R.P. and of Protamine Zinc insulin. This was, of course, a tentative idea, and it was necessary to submit it to experimental test.

Auguste Loubatiéres, in *Annals of the New York Academy of Sciences,* Vol. 71, p. 4.

Maternal Prediabetes as a Cause of the Unexplained Stillbirth

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Before becoming overtly diabetic, women may betray their prediabetic state by producing stillbirths and overlarge babies. We found the stillbirth rate in prediabetic women in the Cape of Good Hope to be 14 per cent, rising to 29 per cent in the five-year period directly preceding diabetes.[1] It is to be expected, then, that a number of stillbirths for which the pathologist can find no good cause may actually be related to latent or prediabetes in the mother. In a preliminary investigation into the glucose tolerance of mothers with babies of various birth weights, eighteen mothers whose last pregnancies had produced unexplained stillbirths were included. Seven of these were found to be mildly but definitely diabetic. Five others had slightly abnormal tolerance curves, with high figures for the two or two and a half hour reading.[1] We gave reasons for considering such curves to be "prediabetic" in type. After being followed up for three years or more all these five mothers became plainly diabetic.[2]

LARGE PANCREATIC ISLETS

Diabetes or prediabetes in the mother may be suspected or even assumed by the finding of enlarged islets of Langerhans on examination of the pancreas of the stillborn infant. It has long been known that the infant of the diabetic mother has large islets,[3,4] and it has more recently been suggested that islet hypertrophy may be present in the infant of a mother who is not at the time diabetic but who becomes so later.[5] We have been able to confirm this. Pancreases from 109 autopsies* which had been performed in this medical school have been examined. Suitable stained sections were projected onto paper of uniform weight and thickness, tracings of the islets were made (figure 1), cut out and weighed. (Method described in detail elsewhere.)[6] In pancreases of stillborn infants in which there was no Rh incompatibility and whose mothers were not known diabetics, the mean islet area of eighty-eight unselected cases was 2.13 per cent (standard deviation 1.7 per cent). Three proved cases of erythroblastosis fetalis had islet areas of over 5 per cent and in three infants of known diabetic mothers the figure was 6.5 to 7.4 per cent (figure 2).

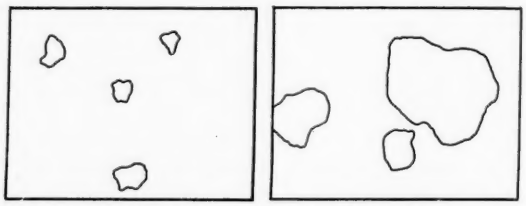

FIG. 1a. Typical tracing of projected islets of Langerhans in case of a stillbirth with a normal mother (left).
1b. Tracing in case of a stillbirth with prediabetic mother (becoming diabetic four years later).

Eighteen additional cases were indexed in the histological files as showing islet hypertrophy. These were also re-examined and the islet areas were assessed.

Large islet area was shown to be unrelated to fetal

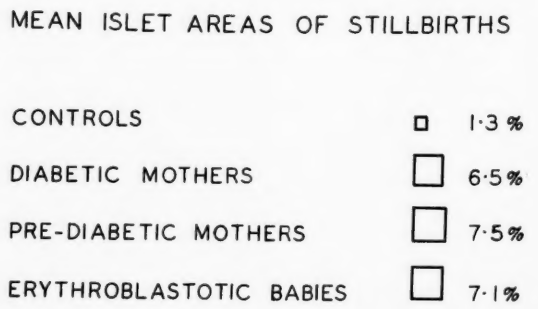

FIG. 2. The 1.3 per cent for "controls" islets is the mean figure for islet area in stillbirths for which there was an obvious cause of death which was neither erythoblastosis nor maternal diabetes.

Presented by title at the Eighteenth Annual Meeting of the American Diabetes Association in San Francisco, June 21-22, 1958.

Endocrine Research Laboratories, Department of Medicine; and Department of Pathology, University of Cape Town, South Africa.

*These autopsies included the stillborn infants and also those who died within forty-eight hours of birth.

body weight or to length of gestation.[6] The problem then was to assess its relationship to carbohydrate tolerance in the mother. After elimination of the three cases of erythroblastosis and the three infants born to known diabetics, there were eighteen with islet areas above 5.5 per cent. (The figure of 5.5 per cent was chosen because this was twice the standard deviation above the mean of 2.13 per cent.) We were able to trace the mothers of twelve of these infants and have performed glucose tolerance tests on them, in some cases on several occasions. Five of these women who, during the pregnancies which produced these abnormal babies, showed no evidence of impaired carbohydrate tolerance, have since become overt diabetics. Five other women had abnormal curves of the type we associate with prediabetes, either in the last trimester of pregnancy or in the immediate postpartum period. On follow-up, two of these five have become more plainly diabetic (figures 3 and 4).

The final two of these twelve mothers had normal tolerance curves on three separate occasions, but in each there was collateral evidence of prediabetes. One had a diabetic mother, uncle and aunt, and had had multiple

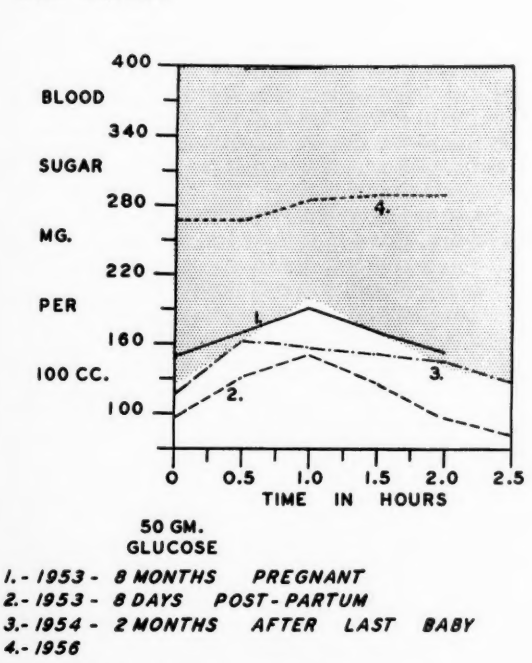

FIG. 3. Glucose tolerance curves on W.S.
(1) Eight months pregnant.
(2) Eighth day after large stillbirth which had hypertrophied islets (13.5 per cent of total pancreas).
(3) One year later, following a live 13-lb. baby.
(4) Three years later, aged thirty-eight.

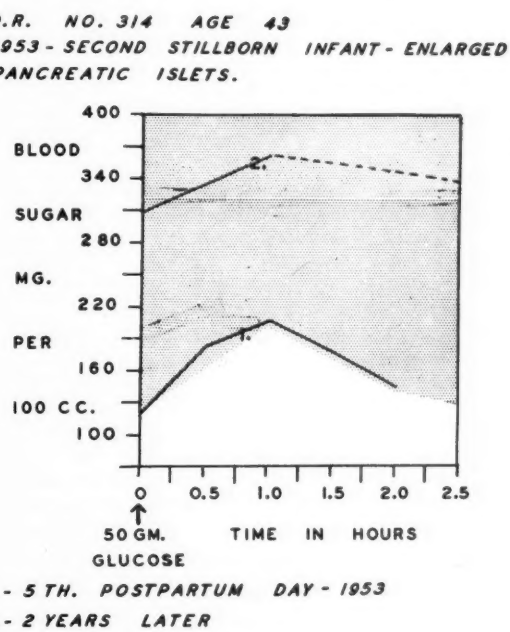

FIG. 4. Glucose tolerance curves in D.R.
(1) On fifth postpartum day after delivery of stillborn infant with large pancreatic islets (12.5 per cent of total pancreas); curve is possibly just diabetic.
(2) Two years later; patient aged forty-three.

stillbirths, while the stillborn child of the second* weighed 14½ lb. We have evidence, therefore, that all twelve mothers of the stillborns with large islets were prediabetic.

We were able to obtain for follow-up seven of the eighty-three mothers of stillborn infants with normal islet area, and all of these had completely normal glucose tolerance curves. Since the expected incidence of diabetes mellitus in the childbearing population is approximately 2.5 per 1,000,[7] we believe that the incidence of impaired carbohydrate tolerance among the mothers of infants with islet hypertrophy is far too high to be coincidental, and that the results justify the estimation of islet area as a diagnostic tool.

THE UNEXPLAINED STILLBIRTH

Among the stillbirths with large islets the pathological cause of death was listed as pneumonia in four, multiple congenital defects in three, cerebral hemorrhage in two, hyaline membrane in one. In eight cases no satisfactory pathological cause of death could be found. We believe

*This patient has recently become overtly diabetic, with symptoms.

TABLE 1

Length of prediabetic period. An over-all stillbirth rate of 14 per cent in women before becoming diabetic, rising to 29 per cent in the five-year period immediately before.

Years Before Diabetes	Total Babies	Babies Over 10 lb. Per Cent	Still-births. Per Cent
0-4	35	54	29
5-9	55	44	13
10-14	61	23	23
15-19	92	28	13
20-29	102	35	8
Over 30	43	26	9.3
Whole series	398	33	14
Control series	439	4.6	5

that the examination of the islets of Langerhans supplies the answer (or at least takes us back one stage further and gives us a clinical correlation). Enlargement of the islets (sometimes rather "continents") of Langerhans, with known maternal diabetes and Rh incompatibility excluded, appears to indicate prediabetes in the mother. Even in cases where a pathological diagnosis has been made, prediabetes may still be the important factor which predisposed to the condition found (e.g., in hyaline membrane disease).

The discovery of such islets and the consequent diagnosis of prediabetes in the mother is not purely a matter of academic satisfaction, but, like the finding of Rh incompatibility, may indicate how to obtain a live baby from the mother's next pregnancy. Such mothers should be treated as though they were established diabetics from the point of view of their confinements, with premature induction of labor and special care of the newborn infant.

SUMMARY

Women in their prediabetic years frequently produce stillborn infants. It is to be expected, therefore, that some unexplained stillbirths may be related to maternal prediabetes. We purport to show that a presumptive diagnosis of prediabetes in the mother may be made from the finding of hypertrophied islets of Langerhans in the stillborn pancreas, provided erythroblastosis can be excluded. In eight cases in our series we have been able to offer this explanation of the cause of an unexplained stillbirth, while in several others the maternal prediabetic state probably played a part in predisposing to the pathological condition which was found.

SUMMARIO IN INTERLINGUA

Prediabete Materne Como Causa De Alteremente Non-Explicate Nascentia Morte

Feminas frequentemente parturi morte-natos durante lor annos prediabetic. Ergo il pare plausibile cercar un relation inter "inexplicate" nascentias morte e prediabete materne. Nostre objectivo es monstrar que un diagnose de presumption de prediabete in le matre pote esser facite super le base del constatation de hypertrophiate insulas de Langerhans in le pancreas del morte-nato, providite que erythroblastosis pote esser excludite. In octo casos in nostre serie il esseva possibile citar iste constatation pro explicar un "inexplicate" nascentia morte, durante que in plure altere casos le stato prediabetic del matre esseva probabilemente un factor in predisponer le organismo al condition pathologic que esseva trovate.

ACKNOWLEDGMENT

We should like to thank Prof. J. G. Thomson for his valuable assistance. Costs were partly defrayed by a grant from the South African Council for Scientific and Industrial Research. Figure 1 is used by permission of the Editor of the *Lancet* and figure 4 by permission of the Editor of the *New England Journal of Medicine*.

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Effects of Insulin, Tolbutamide and Phenethyldiguanidine on Peripheral Glucose Uptake in Man

John Butterfield, B.M., M.R.C.P., Ian Kelsey Fry, B.M., M.R.C.P., and Edward Holling, M.B., F.R.C.P., Philadelphia

In studying carbohydrate metabolism, many investigators have assumed that the arteriovenous, or capillary-venous, blood sugar differences accurately reflect peripheral uptake of glucose, without measurements of blood flow. In many instances[3-4] such arteriovenous studies were performed before reliable methods for estimating peripheral blood flow were available.[5,6] Even recently, with some exceptions,[7,8] measurements of blood flow simultaneous with sampling have been omitted.[9-11] In our view many of these studies are of limited value, since it is recognized that the blood flow through the limbs varies from minute to minute, sometimes by a large factor.[5]

We have therefore developed a technic, similar in many respects to that described by Andres, Calder and Zierler,[8] for the investigation of peripheral glucose uptake. It involves measuring not only small arteriovenous blood sugar differences with accuracy, but also measuring blood flow of the part under study. We have used it in diabetic and nondiabetic subjects to follow the effects of insulin, tolbutamide (Rastinon) and phenethyldiguanidine (PEDG or DBI) on tissue glucose uptake.

METHODS

A full description and evaluation of the experimental and chemical methods used is being presented elsewhere.[12]

From the M.R.C. Clinical Research Unit and the Department of Chemical Pathology, Guy's Hospital, London, S.E. 1, and the Vascular Section of the Robinette Foundation, Hospital of the University of Pennsylvania, Philadelphia 4, Pennsylvania.

This paper is based on material presented at the Third Congress of the International Diabetes Federation at Düsseldorf, July 21-25, 1958. The studies were carried out with the assistance of Caroline Hooper, Annette May, and Margaret Whichelow.

Requests from Europe for reprints may be addressed to Dr. W. J. H. Butterfield, D. M. Guy's Hospital, London, S.E. 1, and from America to H. Edward Holling, M.D., Robinette Foundation, Hospital of the University of Pennsylvania, 3400 Spruce Street, Philadelphia 4, Pennsylvania.

In the present investigations the subjects fasted overnight (sixteen hours) during which time any insulin treatment was omitted. Studies were made on a hospital ward, the subjects lying comfortably in bed.

Figure 1 shows the apparatus. Fine polythene catheters were threaded through needles into the brachial artery and into an antecubital vein carefully chosen as draining the muscle compartment of the forearm.[13] The catheters were kept open by the very slow infusion of heparinized saline. When blood samples were withdrawn special care was taken to avoid contamination with the saline.

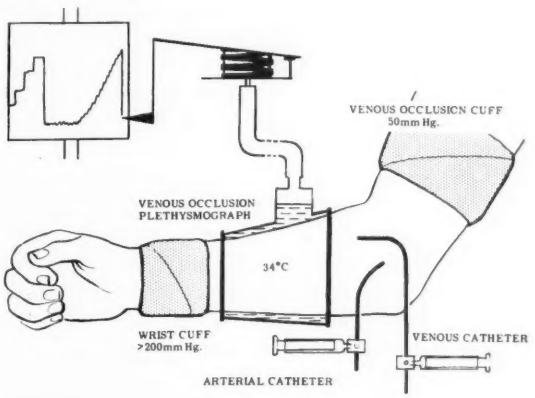

FIG. 1. Apparatus showing the arrangements to measure blood flow, and sample arterial and venous blood.

Blood flow was measured with a venous occlusion plethysmograph. This was always done just before and immediately after (and sometimes during) the withdrawal of blood samples.

Six to eight tenths milliliters of blood were withdrawn from the artery and vein simultaneously, and at the same rate, over a period of one minute. Weighed aliquots of this blood[12] were subjected to duplicate analysis for glucose, using glucose oxidase.[14] Samples were drawn at four-minute intervals.

After taking four fasting samples, 7 gm. glucose (30 per cent) was given intravenously over twenty minutes. This small dose was used to keep the release of endogenous insulin at a minimum.

The peripheral glucose uptake was calculated by multiplying the arteriovenous glucose difference by the blood flow, using the equation: Tissue glucose uptake = Blood flow × arteriovenous glucose difference. The units used for tissue uptake were mg./100 ml. forearm/minute. It should be noted that tissue uptake so calculated does not differentiate between extra- and intracellular compartments.

RESULTS

1. *Tissue glucose uptake in normal and diabetic subjects*

We were interested in establishing what happens to glucose uptake when the arterial blood glucose level is raised by giving small quantities of glucose to fasting subjects.

Figure 2 shows the results when glucose is given to a nondiabetic male and to an insulin-dependent male diabetic sixteen hours after his last dose of soluble insulin.

The lower curve is derived from results obtained in the control subject. It will be seen that in both instances there is a direct, apparently linear, relationship between arterial glucose level and tissue uptake; as the arterial glucose rises, uptake increases. Note particularly that there is a level of arterial blood sugar which must be exceeded for glucose to leave the blood. We define this level as the tissue glucose "threshold." In studies on six control subjects and six mild diabetics, not requiring insulin, we usually found a fasting arterial blood sugar 4 to 5 mg. in excess of this threshold.[12] These persons therefore show a small positive glucose uptake in the fasting state, results which are in agreement with those reported by Andres et al.[8]

The upper curve shows the relationship between arterial blood sugar and tissue glucose uptake in a typical insulin-dependent, thin ketotic-type diabetic sixteen hours after withdrawal of insulin. The obvious and important difference from the normal is that the curve has, as it were, moved upward; the tissue threshold in this patient was 286 mg./100 ml. Such elevated thresholds have now been found in all of the twenty-two diabetics studied so far.

2. *Effects of insulin on tissue glucose threshold*

Figure 3 exemplifies the effect of insulin on an abnormally high threshold. The upper curve is that already shown for the ketotic insulin-sensitive diabetic in figure

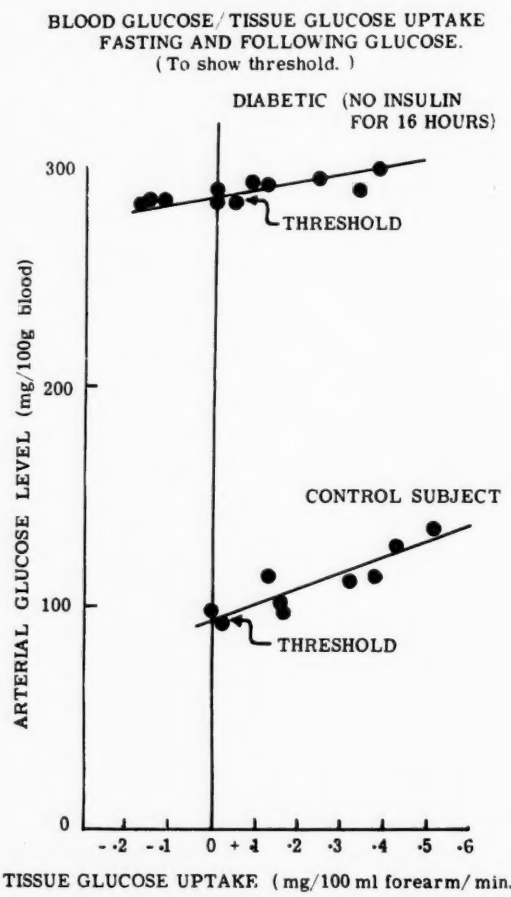

FIG. 2. Relationship between arterial blood sugar and tissue glucose uptake (a) below, in normal subject and (b) above, in an insulin-dependent diabetic patient. The arterial blood sugar was raised by injecting 7 gm. glucose intravenously over a period of twenty minutes. Note threshold levels, where curves cross line for zero uptake. Negative AV glucose differences such as those shown above are discussed elsewhere.[12]

2. The lower curve is that obtained when the same patient had been given 30 units of glucagon-free crystalline insulin, subcutaneously, two hours before the same dose of glucose. It shows that the tissue threshold was reduced to 110 mg./100 ml. and any rise of arterial glucose level was again associated with an increased tissue glucose uptake. Furthermore, the relationship between rise of arterial glucose and rise of glucose uptake was apparently unaltered by insulin. Similar results have been obtained in three other patients.

The effect of insulin on the tissue glucose threshold can be studied more specifically by injecting 0.1 units of glu-

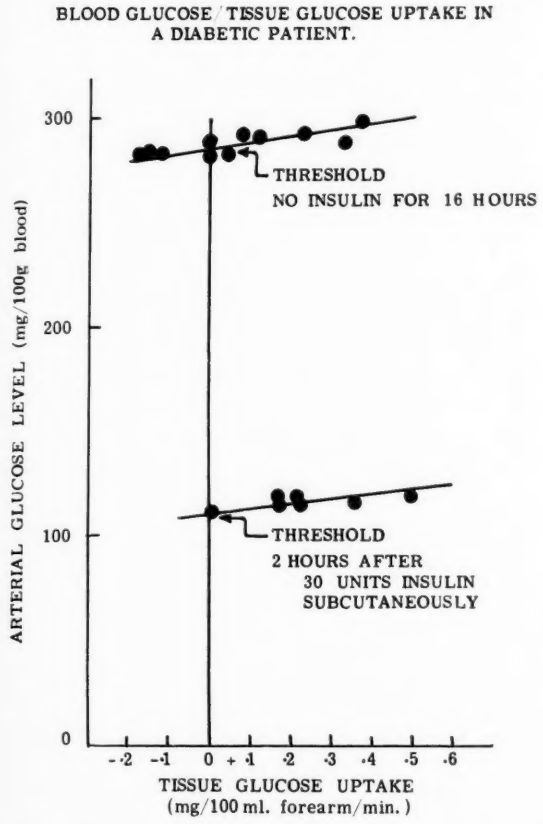

FIG. 3. Effect of insulin on relationship between arterial glucose level and tissue uptake (a) no insulin for sixteen hours, and (b) two hours after subcutaneous injection of 30 units soluble insulin.

cagon-free crystalline insulin slowly into the brachial artery and following the subsequent effect on tissue uptake. Figure 4 shows typical results in a fasting thin male diabetic. The curve shows the results of calculations of the tissue glucose threshold. It will be seen that the threshold begins to fall shortly after the injection and that it continues to fall for some thirty to forty minutes. Similar findings have been made in three other patients, in whom the dose of insulin caused no significant reduction of blood sugar.

3. *Effects of tolbutamide and phenethyldiguanidine* (DBI) *on tissue glucose threshold*

Our investigations have shown that the oral hypoglycemic agents, tolbutamide and DBI, also have a threshold-lowering effect.

The results obtained in an elderly, plump, female diabetic maintained in hospital on a fixed diet, but no

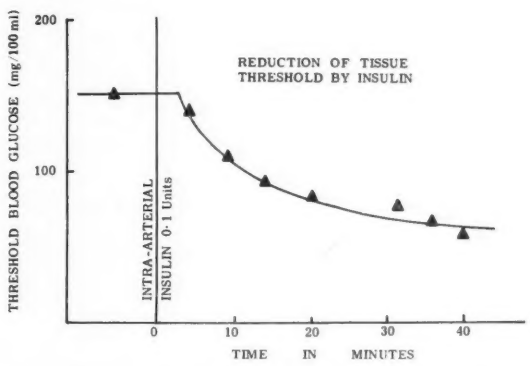

FIG. 4. Tissue glucose thresholds following intra-arterial injection of 0.1 units glucagon-free insulin over two minutes. The threshold at various times after the injection is obtained by drawing through each point a curve parallel to the initial curve, the threshold being taken where each curve crosses zero uptake. It is assumed that the curves for different threshold remain parallel as in figure 3.

insulin, are shown in figure 5. The upper curve refers to the results before the patient was treated with tolbutamide. The tissue threshold was 196 mg./100 ml. The lower curve represents the state of affairs found in a similar test performed after nine days on tolbutamide, by which time the patient had shown an excellent therapeutic response. The tissue threshold was reduced to 77 mg./100 ml. A similar reduction in threshold has been found in three other patients who responded to tolbutamide.

Figure 6 shows the results on an obese, elderly, female diabetic on a fixed diet, before and after successful treatment with DBI. The upper points refer to the results of four samples drawn in the fasting state before DBI. It will be seen that the glucose threshold is about 242 mg./100 ml. After DBI, 50 mg. three times a day for eight days, had produced a satisfactory reduction of blood sugar and disappearance of glycosuria, the fasting blood sugar had fallen and the threshold was 129 mg./100 ml., as shown by the lower curve.

Similar reductions of tissue glucose threshold after oral hypoglycemic therapy have been confirmed in three other patients who responded to such therapy. In our experience these reductions of threshold occur only in those patients in whom oral antidiabetic drugs are successful. Table 1 shows the results of the fasting uptake in three patients who did not respond to the maximum tolerated doses of DBI. All three cases were insulin-dependent diabetics. It will be seen that the tissue threshold was not reduced in any of these nonresponsive cases.

TABLE 1

Tissue threshold in three patients who did not respond to DBI

Case	Age	Sex	Duration of DBI Therapy	Threshold mg./100 ml. blood Before DBI	After DBI
1	22	M	9 days	390	450
2	37	M	8 "	360	470
3	29	F	8 "	240	350

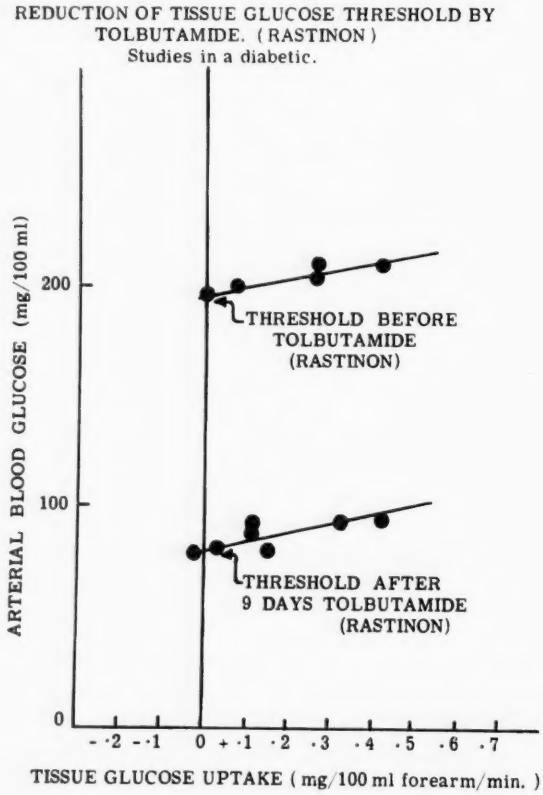
REDUCTION OF TISSUE GLUCOSE THRESHOLD BY TOLBUTAMIDE. (RASTINON)
Studies in a diabetic.
ARTERIAL BLOOD GLUCOSE (mg/100 ml)
200
100
0
THRESHOLD BEFORE TOLBUTAMIDE (RASTINON)
THRESHOLD AFTER 9 DAYS TOLBUTAMIDE (RASTINON)
- ·2 - ·1 0 + ·1 ·2 ·3 ·4 ·5 ·6 ·7
TISSUE GLUCOSE UPTAKE (mg/100 ml forearm/min.)

FIG. 5. Relationship between arterial glucose level and tissue glucose uptake before and after tolbutamide.

DISCUSSION

It seems to us that these results provide new insight into carbohydrate metabolism, diabetes and the mechanism of action of hypoglycemic drugs. We have shown that there is a critical level of arterial blood sugar which must be exceeded before glucose will enter the tissues; this level we have called the tissue glucose "threshold." We have also shown that this threshold approximates closely to the fasting blood sugar, as might be expected if the blood sugar settled during the night towards equilibrium with a tissue glucose threshold.

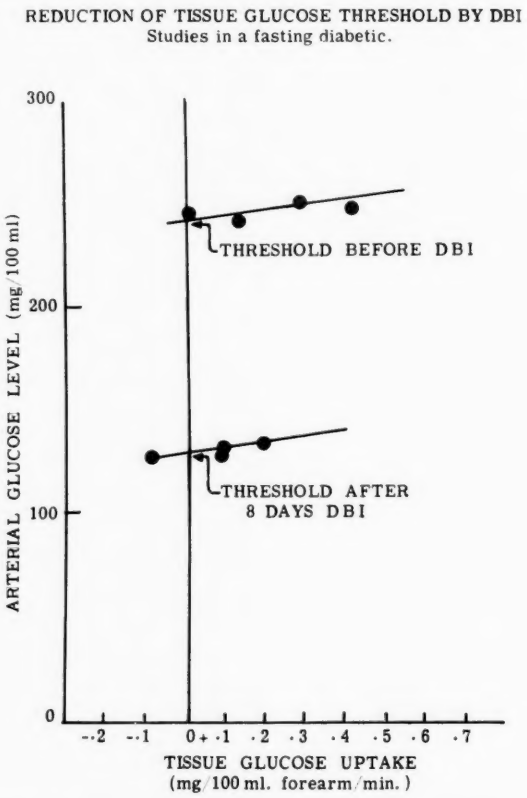
REDUCTION OF TISSUE GLUCOSE THRESHOLD BY DBI
Studies in a fasting diabetic.
ARTERIAL GLUCOSE LEVEL (mg/100 ml)
300
200
100
0
THRESHOLD BEFORE DBI
THRESHOLD AFTER 8 DAYS DBI
- ·2 - ·1 0 + ·1 ·2 ·3 ·4 ·5 ·6 ·7
TISSUE GLUCOSE UPTAKE (mg/100 ml. forearm/min.)

FIG. 6. Relationship between arterial glucose level and tissue glucose uptake before and after phenethyldiguanidine.

Our findings also show that insulin lowers the threshold, presumably by enhancing the permeability of the cell membrane to glucose. This is in accordance with general concepts about the peripheral action of this hormone.[15,16] It should be pointed out that our evidence to date suggests that insulin does not alter the relationship between rise of blood sugar and increase of uptake; that is to say, the curves in figure 3 are more or less parallel. This implies that the primary action of insulin in the peripheral tissues is a reduction of tissue glucose threshold. Any such reduction of threshold will, of course, tend to increase the tissue glucose uptake for any particular glucose level.

The changes of threshold after an intra-arterial insulin injection, plotted in figure 4, are based on extrapolations assuming that the parallelism mentioned above also obtains under these circumstances. The results indicate that insulin injected into the brachial artery is absorbed (fixed) by the tissues from the blood stream during its

single brief passage through the vascular bed of the forearm. From figure 4 it would appear that the action of insulin in lowering the tissue glucose threshold begins very soon thereafter (minutes), and continues for up to half an hour or so after the injection, by which time the threshold seems to have stabilized at a lower level. The timing of events shown in figure 4 agrees well with the rate of fall of blood sugar observed after intravenous injections of insulin into fasting subjects. Under these latter circumstances, it may be postulated that a generalized reduction in tissue threshold is brought about by insulin and this accounts for the fall in blood sugar. By contrast, from the high thresholds seen in diabetic patients, it may be inferred that, in the absence of adequate supplies of insulin, the threshold rises, with a consequent elevation in the level of blood sugar.

Regarding the oral hypoglycemic compounds, whatever hypothesis may be favored as an explanation of their mechanism of action, all investigators seem agreed about one alteration of patients' glucose metabolism after successful treatment, namely that there is a lowering of the fasting blood sugar level.[17–20] We have shown that these drugs lower the tissue glucose threshold; in this their effect is similar to that of insulin.

Further studies are needed to decide whether the effect of oral hypoglycemic agents on the tissue glucose threshold is a primary action or secondary to their stimulating the release of insulin. As it is observed only after successful treatment, we deduce that it is an important factor in the mechanism of action of these drugs.

SUMMARY

Measurements of arteriovenous glucose differences with simultaneous blood flow estimations have permitted us to compute peripheral glucose uptake. We find that in each subject there is a "threshold" which the glucose concentration of the arterial blood must exceed if glucose is to leave the blood stream. This threshold is raised in patients with diabetes. Insulin lowers the threshold, as does successful oral hypoglycemic therapy with tolbutamide and phenethyldiguanidine.

The significance of the findings in clarifying our understanding of carbohydrate metabolism is discussed.

SUMMARIO IN INTERLINGUA

Le Effectos De Insulina, Tolbutamido, E Phenethyldiguanidina Super Le Acceptation Peripheric De Glucosa In Humanos

Mesurationes del differentias arterio-venose de glucosa in conjunction con estimationes simultanee del fluxo de sanguine ha permittite a nos computar le acceptation peripheric de glucosa. Nos trova que il existe pro omne subjecto un "limine" que debe esser excedite per le concentration de glucosa in le sanguine arterial ante que glucosa pote quitar le circulation sanguinee. Iste limine es elevate in patientes con diabete. Le limine es reducite per insulina e etiam per un efficace oral therapia hypoglycemic con tolbutamido e phenethyldiguanidina.

Le signification de iste constationes in clarificar nostre comprension del metabolismo de hydratos de carbon es discutite.

ACKNOWLEDGMENT

We are indebted to Dr. Ronald Britton of Horlicks Ltd. for supplies of tolbutamide (Rastinon, Hoechst) and to Dr. Wilfrid Simpson of Duncan Flockhart Ltd., for supplies of phenethyldiguanidine (DBI, U.S. Vitamin Corp.) and to Mr. E. A. R. Cook of Eli Lilly Ltd., for supplies of glucagon-free insulin.

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Phosphate Activity in White Cells

Histochemical technics make it possible to determine alkaline phosphatase activity in the white cells. M. Wachstein (*J. Lab. & Clin. Med. 31*:1, 1946) demonstrated that the alkaline phosphatase activity of the white cells is almost completely confined to the mature segmented neutrophils. Normal polymorphonuclear leucocytes contain only small amounts of this enzyme, but the activity increases considerably in acute infections and after trauma, hemorrhage, or infarction. In chronic myeloid leukemia, Wachstein found little or none of this enzymatic activity in the white cells.

Recently, B. J. Leonard, M. C. G. Isaels and J. F. Wilkinson (*Lancet 1*:289, 1958) have reported a modification of the original histochemical technic, which offers a simple method of determining the alkaline phosphatase activity in the blood or bone marrow smears.

The role of the phosphatase in the metabolism of the white cell is not known at present, although it has been noted that a modification in the alkaline phosphatase activity in the cells of patients with chronic myeloid leukemia appears to precede the development of the definite cytological abnormality. Since there is an increase in the alkaline phosphatase activity in the white cell following various types of stress (e.g. hemorrhage, infection, trauma, and the like), it has been suggested that this may also be another effect controlled by the pituitary-adrenal glands. Also, the administration of cortisone or ACTH has been noted to increase the phosphatase activity of the white cells. Should adrenal activity be reduced (such as in Addison's disease) the administration of cortisone will increase the phosphatase activity of the white cells, but ACTH will not. However, as yet there has been no definitive evidence to indicate that there is a definite control of the white cell alkaline phosphatase activity by either the adrenal or pituitary glands.

From *Nutrition Reviews,* Volume 16, Number 7, 1958, pp. 222-23.

A New Diagnostic Procedure for Mild Diabetes Mellitus

Evaluation of an Intravenous Tolbutamide Response Test

Roger H. Unger, M.D., and Leonard L. Madison, M.D., Dallas

A new parameter for the diagnosis of mild diabetes mellitus would be a valuable supplement to the various types of glucose loading tests which constitute the usual method for the estimation of pancreatic beta-cell function. The most commonly used of these diagnostic procedures is the standard oral glucose tolerance test, which, although sensitive[1] when interpreted by criteria of the American Diabetes Association,[2] is considered by many to be variable and diagnostically unreliable.[1,3–8] In a recent study[1] the prevalence of "abnormal" oral glucose tolerance curves in a random population sample was found to exceed by at least fifteen times the estimated prevalence of diabetes in the general population.[9] This evidence of nonspecificity is supported by the results of long-term follow-up of individuals with "abnormal" curves[8] and indicates that rigid adherence to these criteria can lead to over-diagnosis of diabetes.

Since the nonspecificity of the oral glucose tolerance test has been attributed, at least in part, to variable rates of glucose entry from the gastrointestinal tract,[3,10,11] a variety of intravenous glucose tolerance tests, differing from each other in respect to rate of glucose injection, have been employed,[11–18] and appear to offer greater accuracy and reproducibility. Those procedures requiring slow infusion of glucose solution at a constant rate[11,16] are not widely used for routine purposes. Rapid intravenous glucose tolerance tests[13,14,17,18] are simpler to perform and, according to Amatuzio, Stutzman, Vanderbilt and Nesbitt, provide a high degree of accuracy in the differentiation of the nondiabetic and the mildly diabetic state.[18] In an attempt to reduce the number of blood specimens required for the time-disappearance curves employed in such tests, Lozner, Winkler, Taylor and Peters recommended a diagnostic criterion for a single blood glucose determination two hours after injection.[17] Unfortunately the test, when interpreted according to this standard, has been found relatively insensitive, most diabetics with normal fasting blood glucose levels falling within the normal zone.[19] The need for additional accurate but practical diagnostic parameters for mild diabetes mellitus is therefore apparent.

In 1956 Mirsky, Diengott, and Dolger[20] reported that moderately severe diabetic and nondiabetic subjects differ strikingly in their hypoglycemic response to orally administered tolbutamide. Unger and Madison[21] observed similar differences in response to intravenously administered sodium tolbutamide in mild diabetics and nondiabetics, and suggested that these differences might provide the basis for a new diagnostic test for mild diabetes. A tentative rationale for the use of this compound in differentiating diabetics from nondiabetics stems from evidence,[22–26] as yet not incontrovertible, that the sulfonylureas, like glucose[27–29] induce the release of endogenous insulin.

The purpose of the following report is to evaluate the previously published data referred to above[21] in order to determine the merits of a standardized intravenous tolbutamide response test as a diagnostic procedure for mild diabetes mellitus.

MATERIALS AND METHODS

Seventy-nine mild, stable diabetic patients were chosen from the wards of the Veterans Hospital, from the rosters of the Parkland Memorial Hospital Metabolic Clinic and the Diabetes Detection Unit of the City Health Department, Dallas, Texas. None of these patients was receiving insulin or therapy other than dietary restriction at the time of their selection. They were subclassified according to their pretest fasting blood glucose concentration: Thirty-four subjects had diagnostic

Presented by title at the Eighteenth Annual Meeting of the American Diabetes Association in San Francisco, June 21-22, 1958.

From the Department of Internal Medicine, University of Texas Southwestern Medical School and Veterans Administration Hospital, Dallas, Texas.

Presented in part at the Annual Meeting of the American Federation of Clinical Research, May 1957.

elevations (115 to 184 mg. per cent); forty-five had normal or near-normal levels (below 115 mg. per cent), including twenty-five patients with values of less than 100 mg. per cent. The diagnosis of diabetes in the latter group had been established by a three-hour oral glucose tolerance test interpreted according to the following criteria: peak blood glucose level of 200 mg. per cent or more; two-hour level of 170 mg. per cent or above; three-hour level of 135 mg. per cent or more. These stringent diagnostic criteria were applied in order to exclude from the diabetic group any nondiabetics with borderline abnormalities in glucose tolerance curve.

A control group of 100 subjects was selected from among medical, surgical, and otolaryngological patients at the Dallas Veterans Hospital (eighty-five persons) and from among medical students (fifteen persons). None of these control subjects suffered from any known acute or chronic disease of consequence and in all the oral glucose tolerance test was normal by the criteria of the American Diabetes Association.[2]

A standard preparatory diet containing 300 gm. of carbohydrate per day was prescribed for at least three days prior to testing. After an overnight fast a blood specimen was obtained and a gram of sodium tolbutamide* in 11 cc. of distilled water was administered by vein over a two-minute period. Blood specimens were obtained at various intervals after the mid-point of the injection. Blood glucose determinations were performed in duplicate by the Somogyi-Nelson technic.[29]

RESULTS

The typical blood glucose response curves of each group are illustrated in figure 1.

Comparison of nondiabetic controls and diabetic subjects. After the intravenous injection of sodium tolbutamide nondiabetic subjects exhibited a rapid decline in blood glucose concentration, not unlike that which normally follows the intravenous injection of insulin. At twenty minutes after injection the mean level of the nondiabetic group had fallen to 60 per cent of the pretest value (S.D.±14.2), with a range of 85 to 9 per cent. At thirty minutes the mean glucose concentration reached a nadir of 51 per cent of the pretest level (S.D.±14.8) and the range was from 82 to 6 per cent. The forty-minute value of 55.6 per cent (S.D.±11.9) reflected an increasing prevalence of rebounding glucose levels and at sixty minutes the mean glucose concentration was 72 per cent (S.D.±12.1) of pretest value.

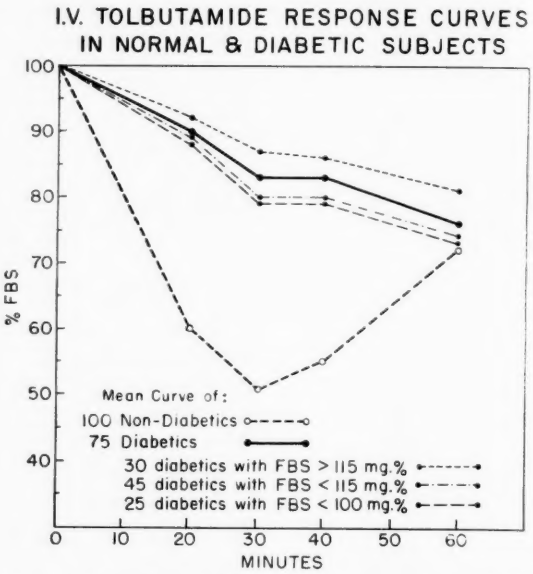

FIG. 1. The mean blood glucose response curves of nondiabetic and diabetic subjects. (The omission of four members of the diabetic group does not appreciably alter the appearance of these curves.) The diabetic group is subdivided according to the level of FBS. The forty-five diabetics with FBS below 115 mg. per cent include those twenty-five patients with FBS below 100 mg. per cent. (Reprinted with the permission of "The Journal of Clinical Investigation," Volume 37, 1958.)

In contrast, diabetic subjects exhibited a far less rapid decline toward a more distant nadir (figure 1). The mean glucose concentration at twenty minutes fell to only 90 per cent of the pretest value (S.D.±7.4) and ranged from 108 to 61 per cent. Thirty minutes after injection the mean level was 83 per cent (S.D.±8.7), and ranged from 105 to 49 per cent. The forty-minute value was 83 per cent (S.D.±9.8), and the sixty-minute value was 77 per cent (S.D.±10.9).

Forty-five members of the diabetic group had fasting blood glucose levels of below 115 mg. per cent and twenty-five of these patients had normal levels (below 100 mg. per cent). It will be noted in figure 1 that the mean tolbutamide response curve of each of these two subgroups parallels that of the more severely diabetic group and is strikingly different from that of the nondiabetic group.

This point is further emphasized in figure 2 which compares the tolbutamide response curves of thirteen nondiabetic controls with those of thirteen mild diabetic patients whose fasting blood glucose concentration was in the same normal range. Despite the similarity of the

*Sodium tolbutamide was generously supplied by Dr. C. J. O'Donovan, The Upjohn Company, Kalamazoo, Michigan.

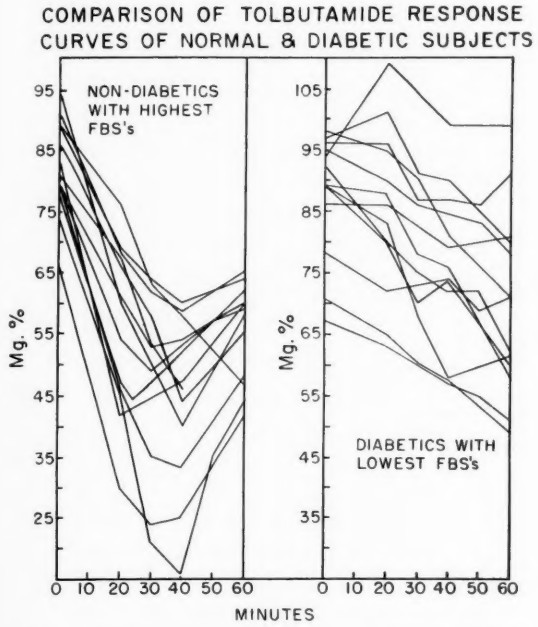

FIG. 2. Tolbutamide response curves of thirteen nondiabetic and thirteen diabetic subjects whose pretest blood glucose levels were in a similar range. The rapid fall characteristic of nondiabetic subjects is in distinct contrast to the more gradual decline typical of the diabetics, and illustrates the ability of the test to detect very mild forms of the disease. (Reprinted with the permission of "The Journal of Clinical Investigation," Volume 37, 1958.)

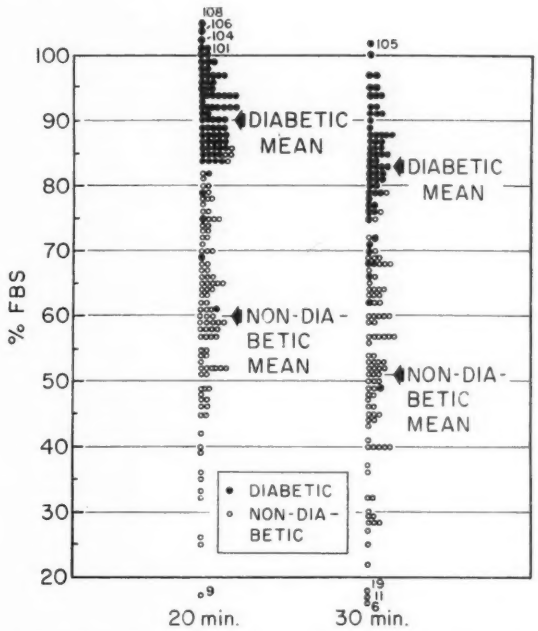

FIG. 3. The blood glucose levels at twenty and at thirty minutes after tolbutamide injection, expressed as per cent of the pretest value, of all diabetic and nondiabetic subjects are recorded.

pretest glucose levels of each group, the precipitous decline of the nondiabetic subjects is in distinct contrast to the more gradually declining curves of the mild diabetics. *Selection of criteria for the differentiation of diabetics and nondiabetics by means of the intravenous tolbutamide response test.* Figure 1 reveals that, of the specimens analyzed, maximum separation of diabetic and nondiabetic response curves took place at twenty and at thirty minutes after tolbutamide injection. These points have, therefore, been selected for closer examination. In figure 3 all twenty- and thirty-minute blood glucose values, expressed as per cent of pretest level, are recorded. It will be noted that at twenty minutes, the glucose level of 96 per cent of nondiabetic subjects had fallen below 84 per cent of pretest level, and to below 80 per cent in 94 per cent of the group. However, the glucose level of 94 per cent of diabetic patients was at 84 per cent of the pretest value or above, and was at 80 per cent or above in 95 per cent of the group.

At thirty minutes after injection, blood glucose concentration of 99 per cent of nondiabetic controls was less than 77 per cent of the pretest level (figure 3). This lower prevalence of "false positive" results at thirty minutes is offset by the observations in the diabetic group. In only 82 per cent of the diabetic subjects did the blood glucose level remain at 77 per cent of the pretest value or above. This increased number of false negative results among the diabetics indicates a seriously diminished degree of sensitivity at this time.

These data indicate that the arbitrary selection of 84 per cent and above as the diabetic zone for the twenty-minute specimen provides a diagnostic standard which has been correct in 95 per cent of the subjects in this study. Similarly, the selection of 80 per cent of the pretest level as the upper limit of the nondiabetic zone for the twenty-minute specimen provides a standard which was accurate in 96 per cent of subjects tested. The zone between 80 and 84 per cent of the pretest level is to be considered to be presumptively abnormal though nondiagnostic.

The blood glucose level at thirty minutes seems far more specific but much less sensitive than that of the twenty-minute specimen. Only the diabetic zone is of importance here, since negative values have little sig-

nificance in excluding the disease. The interpretation of blood glucose levels of 77 per cent and above as diabetic provided 99 per cent accuracy in this series.

In the interpretation of results falling within the zone of overlap, which at twenty minutes extends from 75 to 89 per cent of pretest level and includes 10 per cent of each group, rigid adherence to the recommended criteria can lead to diagnostic errors. Instead, within this zone diagnostic probability must be taken into consideration. According to these limited data persons whose blood glucose level at twenty minutes falls within the 75 to 79 percentage range have a 21 per cent probability of diabetes; those whose level falls between 80 to 84 per cent have a 56 per cent chance of being diabetic; and between 85 to 89 per cent, the diagnostic probability is 91 per cent. Below and above the zone of overlap diagnostic accuracy approaches 100 per cent.

The probability of normality or abnormality of each range of blood glucose response for the twenty- and thirty-minute specimens can be estimated more readily in the frequency distribution charts provided in figures 4 and 5.

Side reactions. Side reactions, due either to drug toxicity or to hypoglycemia, were unusual. Several patients complained of hunger and weakness during the tolbutamide test, but nervousness, trembling, sweating and headache were uncommon. Such symptoms were noted only among nondiabetics, since the blood glucose concentrations of diabetics seldom reached hypoglycemic levels during the test period. Blood glucose concentrations below 25 mg. per cent were recorded in 23 per cent of the nondiabetic subjects, few of whom complained of hypoglycemic symptoms. In several of the patients who reported hypoglycemic symptoms, blood sugar levels ranged well above 25 mg. per cent. In none of these cases was it necessary to terminate the test.

A few patients complained of transitory shoulder pain during the intravenous injection and this was attributed to venospasm. In one individual with a history of drug sensitivity manifested by generalized chronic urticaria, the onset of nausea, vomiting, abdominal pain and an acute exacerbation of urticaria followed within two minutes of the tolbutamide injection and subsided spontaneously within six hours.

DISCUSSION

The foregoing data indicate that the intravenous tolbutamide response test correctly differentiates known diabetic subjects from nondiabetic controls in 95 per cent of instances and appears, therefore, to qualify as a diagnostic test for diabetes. Furthermore, the mean

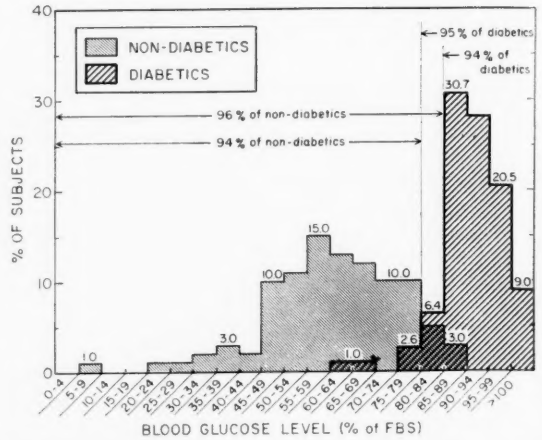

FIG. 4. Frequency distribution of blood glucose levels twenty minutes after tolbutamide injection in 100 nondiabetic and seventy-nine mild diabetic subjects.

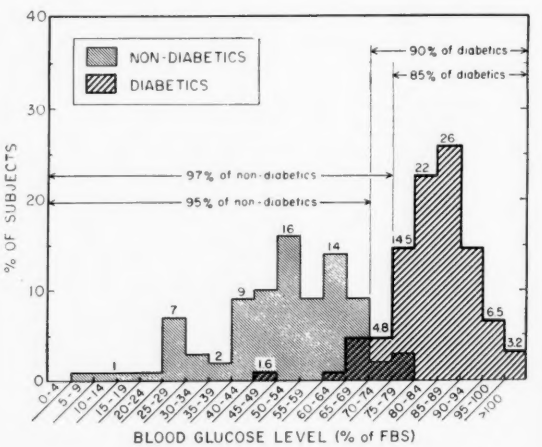

FIG. 5. Frequency distribution of blood glucose levels thirty minutes after tolbutamide injection in 100 nondiabetic and seventy-nine mild diabetic subjects.

tolbutamide response curve of the forty-five mild diabetics with a normal (<100 mg. per cent) or slightly elevated (100 to 115 mg. per cent) fasting blood sugar level closely parallels that of the thirty-four diabetics with fasting hyperglycemia (116 to 184 mg. per cent) of diagnostic proportions, and differs strikingly from that of the nondiabetics. This would indicate a degree of sensitivity sufficient to identify correctly milder forms of the disease.

Diagnostic criteria have been based on the data recorded in figure 3 at the twenty- and thirty-minute specimens, at which time separation of the groups was

maximal. On the basis of the above it is suggested that failure of the blood glucose level to fall below 84 per cent of the pretest level at twenty minutes after injection be considered strong diagnostic evidence of diabetes mellitus. A fall in blood glucose concentration to 80 per cent of the pretest value or below would appear to be strong evidence against diabetes since 96 per cent of such results occurred in nondiabetics. However, a negative result does not entirely exclude diabetes mellitus since the blood glucose level of 5 per cent of diabetics fell below this level. At thirty minutes, failure of the blood glucose level to decline below 77 per cent of the pretest value constitutes even more specific evidence of diabetes. Although of little value in exclusion, this specimen is useful when abnormal, giving added significance to an abnormality at twenty minutes.

The results of an evaluation of a diagnostic test for diabetes will depend to a large degree on the composition of the normal and diabetic groups studied. Our data suggest that if, as has often been done in evaluating other tests, comparison had been restricted to medical student controls (or any similar young population sample) and to overt diabetics, complete separation of the groups would have resulted. However, since the individuals for whom such a test is designed differ markedly from both these categories, evaluations of this type are unrealistic and the standards derived therefrom misleading. For this reason in the selection of the two groups an attempt was made to simulate the population segment most apt to require such a test. In the control group the number of medical students was limited in favor of older nondiabetics, while more than half of the diabetic group were mild cases who, without treatment, maintained normal or near-normal fasting blood glucose levels despite a high-carbohydrate diet.

In any quantitative biologic measurement subjected to large-scale evaluation of this type, a certain amount of overlap between normals and abnormals is likely to appear, the result of skew in the frequency distribution curve of each group. Hence, a zone of overlap between nondiabetics and diabetics is to be expected. Diagnostic errors in this borderline area can be minimized by interpretation in terms of probability of normality or of disease, as has been recommended. Application of these principles to the interpretation of the oral glucose tolerance test, in which overlap between diabetics and nondiabetics appears to be even more extensive,[1] might resolve the controversy[1-8] which now surrounds its diagnostic validity and standards.

In an attempt to confine the evaluation of the tolbutamide test to definite nondiabetics and unequivocal but mild diabetics, stringent and widely separated standards for the interpretation of the oral glucose tolerance test were employed in classification. Unaccounted for, therefore, in these data are those borderline individuals whose oral glucose tolerance curves fall between the criteria used in this study. Because of the uncertain status of this intermediate group, composed, as it appears to be,* of both very mild diabetics and false-positive nondiabetics, a separate study has been underway to determine whether or not the tolbutamide response test can contribute to its proper classification.

Evaluation of the test in the presence of liver disease, endocrinopathies, infection, and carbohydrate restriction remains to be completed. Preliminary data suggest that carbohydrate restriction may result in a "diabetic" tolbutamide response curve. This has also been observed in starved rats,[31] and is consonant with the hypothesis that tolbutamide stimulates endogenous insulin secretion, since it has been shown that fasting reduces pancreatic insulin content.[32] Persons in this study were, therefore, instructed to consume at least 300 gm. of carbohydrate daily during the week before the test.

Among the assets of the intravenous tolbutamide response test is the fact that it is complete within thirty minutes and requires only three blood specimens. Since hypoglycemia of considerable magnitude may occur in certain of the nondiabetics, it is recommended that the test be terminated as soon as the thirty-minute specimen is obtained with the administration of carbohydrate. Although no untoward effects have occurred so far, caution is advisable in the testing of persons with atherosclerosis.

It is tempting to attribute the striking similarity of the normal tolbutamide and insulin response curves to a rapid release of insulin stored in the beta cells. If this were true, the more gradual decline characteristic of mild diabetes might then be ascribed to diminished release of insulin from the beta cells, due either to a deficiency of stored insulin or to a slower rate of release. However, extrapancreatic factors in the serum or at the cellular level might result in a similarly diminished responsiveness to the drug.

SUMMARY AND CONCLUSIONS

Intravenous tolbutamide response tests were performed in 100 nondiabetic controls and in seventy-nine diabetic patients, and highly significant differences in the blood glucose responses of the two groups were observed. Whereas in nondiabetic subjects the blood glucose con-

centration fell sharply to a nadir between twenty and forty minutes after the tolbutamide injection, that of diabetic patients, including those with a normal and near-normal fasting blood glucose level, declined more gradually. Separation of the two groups was maximal at twenty and thirty minutes after the injection. At twenty minutes the blood glucose level of 94 per cent of diabetic patients remained at 84 per cent or more of pretest values, whereas in 96 per cent of nondiabetic subjects it had fallen to 80 per cent or less of the pretest level. These levels have been arbitrarily selected as the standards by which the test can be interpreted with maximum accuracy. The zone between 80 and 84 per cent of the pretest blood sugar level is to be considered as suspiciously abnormal though nondiagnostic. Failure of the blood sugar concentration to decline to below 77 per cent of the pretest value at thirty minutes gives additional weight to abnormalities at the twenty-minute specimen. The use of these criteria has permitted proper identification of diabetics and nondiabetics in the vast majority of subjects tested.

The intravenous tolbutamide response test appears to qualify as a diagnostic test for mild diabetes mellitus and should provide a useful supplement to the various glucose loading tests now used for this purpose. It has been found to be safe, simple, not unpleasant for the patient and has the advantage of requiring only thirty minutes for completion.

SUMMARIO IN INTERLINGUA

Evalutation De Un Test Del Responsa A Tolbutamido Intravenose Como Procedimento Diagnostic In Casos Leve De Diabete Mellite

Tests del responsa a tolbutamido intravenose esseva effectuate in 100 non-diabetic subjectos de controlo e in septanta-nove patientes diabetic. Differentias altemente significative esseva observate inter le duo gruppos in le responsas de lor glucosa sanguinee. In non-diabeticos, le concentration de glucosa sanguinee descendeva abruptemente verso su nadir que esseva attingite inter vinti e quaranta minutas post le injection de tolbutamido. In le caso de patientes diabetic—incluse illes con normal e quasi normal nivellos de glucosa sanguinee in stato jejun—le concentration descendeva plus gradualmente. Le separation del duo gruppos esseva maximal a periodos de vinti e trenta minutas post le injection. Post vinti minutas, le nivello del glucosa de sanguine de 94 pro cento del patientes diabetic remaneva a 84 pro cento o plus del valor pre-experimental, durante que in 96 pro cento del non-diabeticos illo habeva descendite a 80 pro cento o minus del valor pre-experimental. Iste nivellos ha essite seligite arbitrarimente como standards per le quales le test pote esser interpretate con un maximo de accuratia. Le zona inter 80 e 84 pro cento del nivello de sucro de sanguine de ante le experimento debe esser considerate como suspiciosemente anormal ben que non como diagnostic. Si le valor trenta minutas post le injection non es inferior a 77 pro cento del valor pre-experimental, un anormalitate in le valor vinti minutas post le injection gania in signification. Per medio de iste criterios il ha essite possibile identificar diabeticos e non-diabeticos correctemente in le vaste majoritate del subjectos studiate.

Le test del responsa a tolbutamido intravenose es apparentemente qualificate a servir como test diagnostic in leve casos de diabete mellite e promitte devenir un utile supplemento pro le varie tests de cargation con glucosa que es nunc usate pro iste objectivo. Illo se ha monstrate salve, simple, non disagradabile pro le patiente, e distinguite per le avantage que illo require solmente trenta minutas pro su completion.

ACKNOWLEDGMENT

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Since 1942 there has accumulated an increasingly impressive body of scientific evidence indicating that the sulfonylurea compounds are extremely effective hypoglycemic agents in both human and animal subjects. The potential usefulness of these drugs in the management of patients with diabetes has provided the stimulus for innumerable clinical trials. However, the fundamental question regarding the mechanism of this hypoglycemic action has not yet been answered. It is obvious that a full understanding of this mechanism is of more than academic interest. Although many classes of drugs may produce hypoglycemia, the usefulness of such drugs as therapeutic agents in diabetes must be dependent upon more than a hypoglycemic action. There must be, in addition, an enhancement of the over-all metabolism of carbohydrate. In order to evaluate the potential therapeutic benefits that may be derived from the sulfonylureas, it becomes important, first to understand the mechanisms by which hypoglycemia is produced and, second, to measure the certain parameters that might reflect alterations in metabolic processes known to be deranged as a consequence of impaired carbohydrate utilization.

Lillian Recant and George L. Fischer in *Annals of the New York Academy of Sciences,* Vol. 71, p. 62.

Many diabetic patients require dosages of insulin far in excess of what is generally considered the production of the normal pancreas, and in various situations, most strikingly in severe ketosis, the insulin tolerance may increase enormously. Under these circumstances, one must suppose that an "antagonist" to insulin is present in undue amounts in the blood or tissues. Whether this antagonist is in the nature of a pituitary hormone, an antibody, or a specific destructive enzyme (insulinase) remains to be determined.

DeWitt Stetten, Jr., in *Currents in Biochemical Research,* p. 171. Edited by David E. Green. Interscience Publishers, Inc., New York, 1956.

A Quantitative Estimation of Antibodies to Exogenous Insulin in Diabetic Subjects

Pierre Moinat, M.D., with the technical assistance of Ellen Marston, Boston

Enough evidence is now available to indicate that insulin is weakly antigenic. A few years after the discovery of the hormone and its introduction into clinical use, papers were published showing that the protein molecule, even though small, was responsible for the production of antibodies both in man and in experimental animals. Raynaud and Lacroix in 1925 described severe anaphylactic reactions in an elderly diabetic subject following injections of insulin.[1] This was repeatedly confirmed in subsequent years.[2] Furthermore, Raynaud and Lacroix demonstrated that anaphylactic shock could be induced in guinea pigs by injecting 1 ml. of the patient's serum into animals given insulin the day before. They also obtained positive precipitin tests with the same serum although in their hands complement fixation reactions were negative. In 1931, Barral and Roux[3] showed that, in guinea pigs, anaphylactic shock could be induced with purified insulin, indicating that it was not due to protein "impurities" contained in commercial preparations.

Since that time, several other investigators have confirmed the antigenic properties of the insulin molecule. Bernstein, Kirsner and Turner[4] elicited anaphylactic responses to insulin in guinea pigs. Lewis[5] observed a positive Schulz-Dale reaction (contraction of the isolated uterus strip) in guinea pigs sensitized to insulin. By use of the complement fixation test, Wasserman, Broh-Kahn and Mirsky[6] were able to demonstrate antibodies to insulin in six of eleven rabbits given insulin. Lowell and collaborators[7] consistently induced the formation of specific antibodies to insulin in rabbits, as did also Moloney and Coval[8] and Arquilla and Stavisky.[9]

In man, indirect methods have been devised for the demonstration of such antibodies. Banting, Frank and Gairns[10] reported the development of resistance to insulin in a nondiabetic schizophrenic subject following a course of shock treatment. Insulin mixed with blood serum from this patient and injected into mice gave a lower incidence of convulsions than did corresponding control mixtures. Lowell[11] and Yankelowitch et al.[12] obtained similar evidence for neutralization of insulin with blood sera of diabetic subjects showing some resistance to insulin. Other methods have been devised for the same purpose. They include the fluorescent technic of Cohn[13] and the fixation of insulin tagged with I^{131} on insulin antibodies in human serum after electrophoretic separation on paper.[14]

It seems clear, therefore, that insulin is antigenic and that the induced formation of antibodies can be followed both in animals and in man by indirect methods.

MATERIAL AND METHODS

A specific and quantitative method has been devised in this laboratory for the estimation of antibodies against insulin in the serum of diabetic persons and in nondiabetic rabbits. This very sensitive procedure is basically a modification of the passive hemagglutination method described by Boyden[15] and studied more recently by Borduas and Grabar[16] as well as Antoine and Ducrot.[17] Sensitized sheep red blood cells are used as carriers for insulin. They show agglutination when specific antibodies against insulin are present in the serum to be tested.

Fresh sheep blood is immediately mixed with Alsever solution in the proportion of nine parts of blood to one part of solution. The original stock mixture is then stored in the cold room (+4° C.) for further use.

Sensitization of red blood cells. Red blood cells are spun down in a graduated centrifuge tube at low speed (1,000 to 1,200 rpm) from 10 ml. of the original blood. They are resuspended twice in a phosphate buffer at *p*H 7.2. The last supernatant should be clear of any hemolysis. Moreover, enough red blood cells should be siphoned off the tube to bring the last resuspended mixture to an 8 per cent concentration of erythrocytes.

Six milliliters of dilute tannic acid (1/10,000) in the same buffer are added to 6 ml. of the 8 per cent washed suspension of red blood cells and incubated at 37° C. for ten minutes. At the end of the incubation period, the red blood cells are washed once in the phosphate buffer at

From the Baker Clinic Research Laboratory, New England Deaconess Hospital, Boston.

*p*H 7.2 and resuspended in a second phosphate buffer at *p*H 6.4. When spun down again, the sensitized red blood cells are ready to be mixed up with an insulin solution.

Preparation of the insulin solution. One milligram of recrystallized insulin (Lilly) is dissolved in 3 ml. of a 1:100 dilution of rabbit serum in phosphate buffer at *p*H 7.2. This 3 ml. solution is then added to the centrifuged sensitized sheep red blood cells and, after thorough mixing, incubated at room temperature for fifteen minutes. The sensitized treated red blood cells are finally spun down and washed twice with phosphate buffer at *p*H 7.2. Before the last resuspension, a certain amount of red blood cells is siphoned out in order to obtain a final 2 per cent suspension of erythrocytes.

Preparation for the agglutinating process. Twelve agglutination tubes (7.5 x 1 cm.) are placed on a rack. Eight-tenths of a milliliter of a 1:100 solution of rabbit serum in phosphate buffer (*p*H 7.2) is pipetted into the first tube and 0.2 ml. in each of the following eleven tubes. The serum to be tested (0.2 ml.) is then added to the first tube and carefully mixed by successive drawing up into and blowing out of the pipette. The successive dilutions are obtained when 0.2 ml. solution in tube one is transferred successively from one tube to another after thorough mixing. The last tube (No. 12) is to contain therefore 0.4 ml. of a 1:5,120 dilution of the serum to be tested.

When the progressively increasing dilutions have been made, 0.2 ml. of the 2 per cent suspension of the sensitized treated sheep red blood cells is added to each tube and the final mixture gently shaken. The rack containing the covered tubes is finally stored overnight in the cold room (+4° C.) and examination of the agglutination process, if any, done twenty-four hours later.

Estimation of the results. At the end of the twenty-four-hour period, the hemagglutination tubes are withdrawn from the cold room. The bottom of each is then checked with the aid of a concave mirror. When no agglutination has taken place, the red blood cells accumulate as a convex-shaped sediment modeling the bottom of the test tube. If agglutination has occurred, the red blood cells organize in a fringed, often purplish, layer whose aspect is very different from the one of the nonagglutinated sediment. If shaken gently and evenly, the nonorganized sediment will quickly disaggregate in fume-like opacities. On the contrary, agglutinated purplish sediments will release small clusters of cells. These clusters are resistant to several shakings and disappear eventually as the dilutions increase along the twelve tubes, thus permitting an estimate of the concentration of antibodies against insulin.

Precautions. Special attention to a number of points should be paid in order to insure the reproducibility and even the validity of the method. They have been described in the literature already quoted.[16,17]

The glassware should be kept on a special tray protected from dust after careful washing with dichromate sulfuric solution. Soap is to be avoided at every stage. The sheep red blood cells never leave the graduated centrifuge tube; rather, the different solutions are pipetted in or siphoned out with a vacuum pump. In order to detect possible nonspecific cross-reactions, it is imperative that in each experiment, control tests using normal serum (not containing insulin antibodies) be used. Also, the normal rabbit serum employed only for dilution purposes must be inactivated at 56° C. for one-half hour and tested at least once with sensitized treated red blood cells for the same reason. Finally, it is advisable to test each new supply of sheep red blood cells for the presence of naturally occurring anti-sheep blood cell agglutinins.

Normal rabbit serum. With the animal under anesthesia, blood is drawn by puncture of the prepared aorta. After clotting at room temperature, the serum is separated in a refrigerated centrifuge, desensitized at 56° C. for thirty minutes and stored frozen (—20° C.) between each experiment.

Immunization of rabbits. Four normal young rabbits were injected over a six-month period subcutaneously with a 1 ml. emulsion of recrystallized insulin (Lilly Lot No. T-2842). The adjuvant was the one described by Freund[18] and used more recently by Moloney and Coval.[8] The oil mixture (Arlacel-Bayol 1/9)* was homogenized in a Waring Blendor with recrystallized insulin (1 mg./ml.). Phenol 0.3 per cent was also added and the *p*H adjusted between 2.5 and 3.0 by addition of chlorhydric acid. The emulsion was afterwards kept in the cold room.

COMPOSITION OF THE SOLUTIONS

Given here and on the following page are the compositions of the various solutions used:

Phosphate Buffer (Sörensen).

Solution A	KH_2PO_4	1/15 M
Solution B	Na_2HPO_4 $(2H_2O)$	1/15 M

*Specially treated Arlacel A. (Mannide Mono-oleate) Pre. 4B from Atlas Powder Co., Wilmington, Del., and Esso Bayol F. NE-10782-369.

The preceding stock solutions are stored in brown bottles in the dark and preferably in the cold room (4° C.).

Buffer No. 1 (*pH*: 7.2)		*Buffer No. 2* (*pH*: 6.4)	
Solution A	12 ml.	Solution A	28 ml.
Solution B	28 ml.	Solution B	12 ml.
Physiol. Saline (0.9 per cent NaCl)		Physiol. Saline	40 ml.

Alsever solution:

Glucose	2.05 gm.
Sodium citrate	0.8 gm.
Sodium chloride	0.42 gm.
Distilled water to	100.0 ml.

The *p*H is brought to 6.1 with a 10 per cent sodium citrate solution. When freshly prepared, the solution is filtered on a Berkefeld filter and stored in a brown bottle in the dark at +4° C.

Tannic acid solution:

Tannic acid*	1.0 gm.
Phosphate buffer (*p*H 7.2)	100.0 ml.

The stock solution is also stored in a brown bottle in the cold and is diluted to 1:100 with the same buffer immediately before use. It should be discarded when a brownish color persists at the highest dilution.

RESULTS

The formation of antibodies to exogenous insulin, as detected by the method described above, has been studied in rabbits and in diabetic subjects treated with commercial insulin preparations made by Eli Lilly and Company (mixture of beef and pork insulin). In addition, tests were carried out with three sera in which, by other methods, antibodies to insulin had been demonstrated in other laboratories.†

Table 1 shows that three of the four rabbits had titers of antibodies ranging from 1:40 to 1:5,120 at the end of a five- or six-month period of immunization. This is well in accordance with the findings of the authors mentioned earlier.

Table 2 shows the results obtained in three insulin resistant diabetic patients. It can be seen that all of them had appreciable amounts of insulin antibodies as expressed by the high dilution at which agglutination was observed. The history of subject P.L. is of interest. Several members of the family of this twenty-seven-year-old woman had various allergic diseases. The patient herself was allergic to penicillin before the onset of diabetes. The metabolic disease was first discovered in January 1957, and controlled with as little as 10 to 12 units of insulin daily. The dose of insulin had to be progressively increased until 300 units daily were necessary in May and 400 units daily in June 1957. It can also be seen that the titers of antibodies increased in her serum during the period of observation as established with duplicate agglutination tests on the same serum sample.

TABLE 1

Antibodies to exogenous insulin in four normal rabbits

Number	Duration of Immunization	Agglutination Titers
1	6 months	1:40
2	5 "	1:320
3	5 "	None
4	6 "	1:5,120

TABLE 2

Insulin antibodies in diabetic subjects with insulin resistance

Date 1957	Patient	Age in Years	Duration Diabetes in Years	Daily Insulin Dose in Units	Agglutination Titers
May 13	P.L.	27	6/12	300	1:5,120
May 15	P.L.	"	"	"	1:5,120
May 23	P.L.	"	"	"	1:5,120
June 14	P.L.	"	7/12	400	1:10,240
May 28	A.S.	65	15	400	1:5,120
June 6	A.S.	"	"	"	1:10,240
May 9	H.G.	63	10	150	1:320
May 17	H.G.	"	"	280	1:5,120

Hemagglutination tests were performed with the sera of ninety-five diabetic subjects selected at random from the patients of the Joslin Clinic. Most of the results are also based on duplicate determinations. They have been correlated according to the age of the subjects (figure 1), the years of insulin treatment (figure 2) and to the amounts of insulin received daily (figure 3). It will be seen that seventy individuals had positive titers which showed no relationship to age, years of insulin treatment or daily doses of insulin. The twenty-six negative reactions were found in two types of patients, i.e., the newly discovered diabetics[13] under treatment for less than a year and elderly patients[13] with mild diabetes requiring only small doses of insulin daily.

The description of the case of a thirteen-year-old boy is of interest. He had been treated for eight years with 80 units of NOVO insulin daily. His serum failed to show

*Acid Tannic, U.S.P.

†We are indebted to Dr. P. Moloney and Dr. E. Arquilla for providing us with antibody-containing sera.

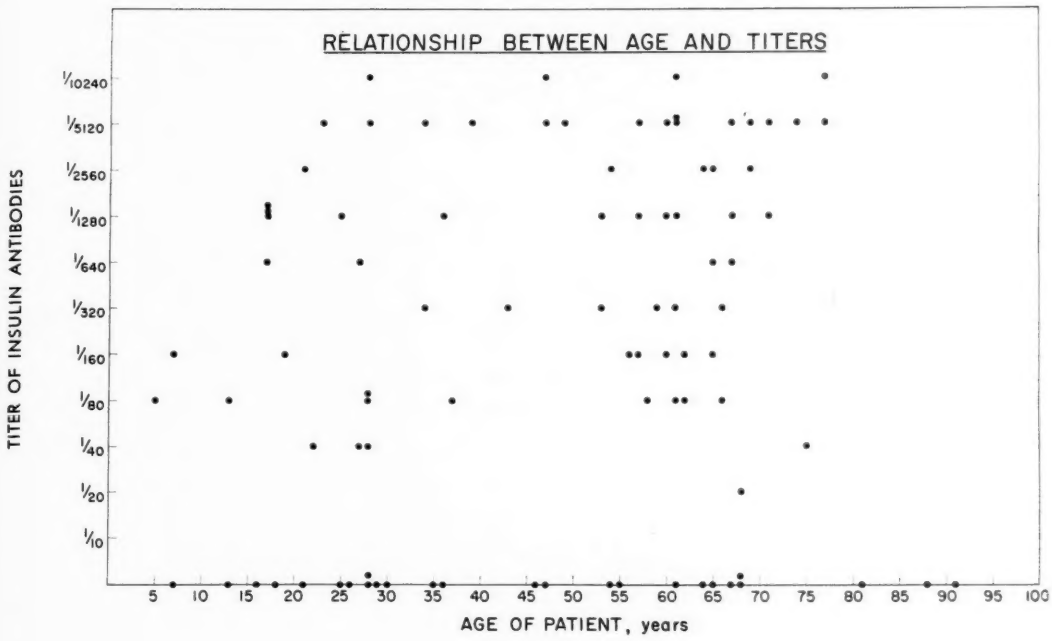

FIGURE 1

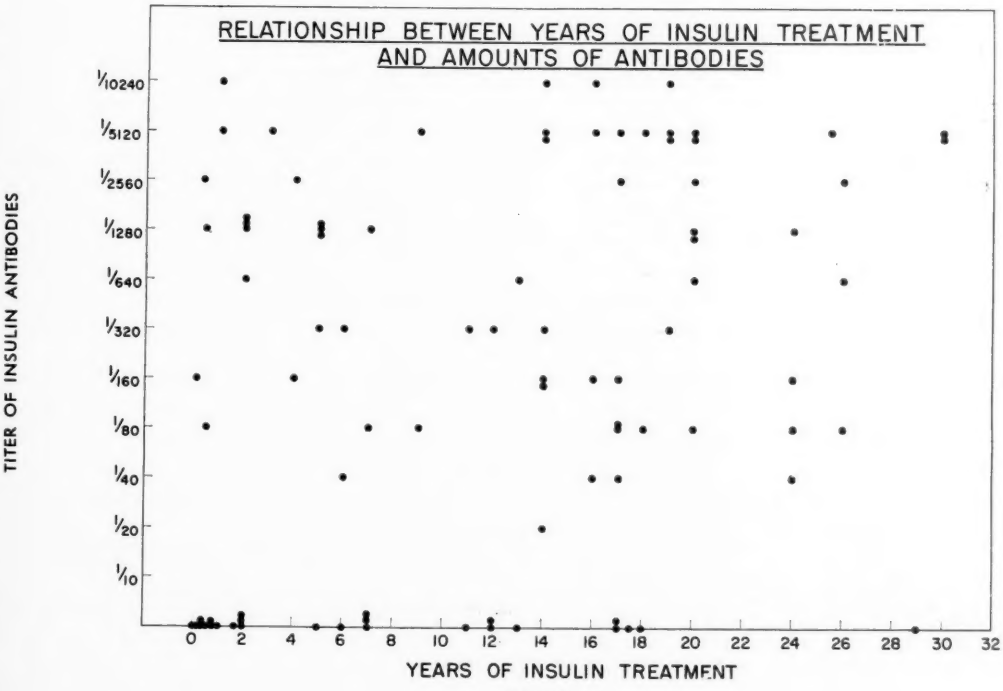

FIGURE 2

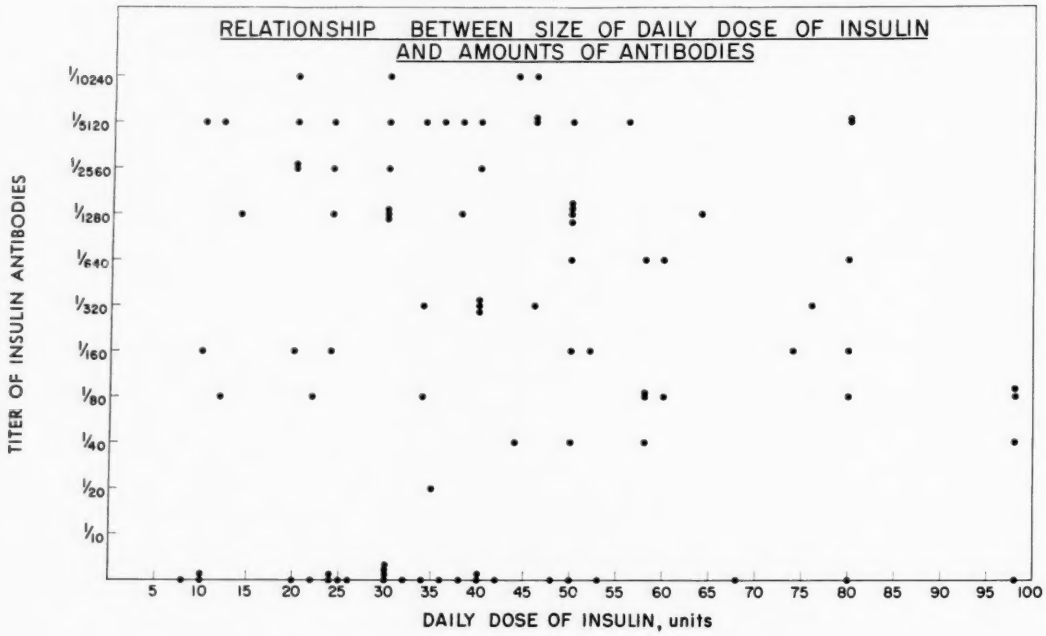

FIGURE 3

any agglutination on repeated examination suggesting that the tested antibodies to the Lilly brand of insulin were missing.

There is a fair agreement today that antibodies are produced by the plasma cells.[19–23] J.W.L., aged fifty-six, was admitted to the New England Deaconess Hospital for multiple myelomas. The diagnosis was based on histological evidence, but the electrophoretic resolution of his serum proteins failed to reveal any sharp characteristic peak in the globulins, save some diffuse increase of the gamma fraction. He had been given 10 units of insulin daily over a two-week period prior to the time his serum was tested for the presence of specific antibodies.

The agglutination was found to be strongly positive up to a dilution of 1:5,120, indicating that abnormally active plasma cells may elicit a quicker response to an antigenic stimulus.

Bio-assays elsewhere (neutralization of insulin by the serum and subsequent injection of the mixture into mice) had shown the presence of specific antibodies in the three sera obtained through the courtesy of Dr. R. Arquilla and Dr. P. Moloney.

Agglutination tests in our laboratory revealed the presence of these antibodies in all of the sera with titers ranging from 1:160 to 1:5,120.

SUMMARY AND CONCLUSIONS

A specific and quantitative procedure has been described for the estimation of antibodies to insulin. Evidence is presented to show that specific antibodies can be induced in rabbits by repeated injections of recrystallized insulin with adjuvant. An antigenic response to the hormone has also been demonstrated in insulin-resistant and noninsulin-resistant diabetic subjects. This response is related principally to the duration of insulin treatment and to some extent to the age of the patient.

The method was used also for the titration of antibody containing sera obtained elsewhere and yielded results in accordance with those established by other methods.

SUMMARIO IN INTERLINGUA

Un Estimation Quantitative De Anticorpore Contra Insulina Exogene In Subjectos Diabetic

Es describite un technica specific e quantitative pro le estimation de anticorpore contra insulina. Es presentate datos provante le possibilitate de inducer le formation de anticorpore specific in conilios per repetite injectiones de insulina recrystallisate in combination con un adjuvante. Un responsa antigenic contra le hormon ha etiam essite demonstrate in subjectos diabetic tanto resistente como etiam non-resistente a insulina. Iste responsa es relationate principalmente al duration del

tractamento con insulina e in un certe mesura al etate del patiente.

Le methodo esseva usate pro titrar seros a contento de anticorpore obtenite alterubi. Le resultatos esseva de accordo con illos obtenite per altere methodos.

ACKNOWLEDGMENT

This work was supported by Research Grant A-525 (C2), National Institute of Arthritis and Metabolic Diseases, U.S. Public Health Service.

We are grateful to Andre Equen, M.D. (Institut Pasteur Paris) for helpful suggestions and useful leadership during the preparation of this work.

Eli Lilly and Company, Indianapolis, Indiana, generously supplied the recrystallized insulin used in these studies.

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The majority of physiological or pharmacological substances are not characterized by a simple specific exclusive mechanism of action. In addition to the principle of preferential action there exist secondary effects which depending upon experimental conditions or species may become more prominent than the principal effect. Sometimes the secondary actions reinforce the primary ones, and sometimes they are in opposition. Under certain circumstances, the interrelationship between the primary and the secondary effects is so integrated that it is difficult to dissociate one from the other.

The hypoglycemic sulfonamides are no exception to this rule; they have both primary and secondary action. The emergence of one or the other depends upon the type of experiment (acute or chronic). It is the object of the physiologist or the pharmacologist to analyze, if possible, to dissociate the observed effects.

Auguste Loubatiéres, in *Annals of the New York Academy of Sciences,* Vol. 71, p. 192.

Clinical Use of the Biguanides and Their Role in Stabilizing Juvenile-type Diabetes

Leo P. Krall, M.D., Priscilla White, M.D., and Robert F. Bradley, M.D., Boston

One year ago we reported the results[1] of clinical trials with DBI at the Seventeenth Annual Meeting of the American Diabetes Association in New York. At that time the conclusions were that the biguanide, N′beta-phenethylformamidinyliminourea hydrochloride (also known as DBI and phenethylbiguanide) had a considerable blood-sugar lowering ability in diabetics of all types and in spite of a relatively high incidence of side effects, showed much promise as a future agent in the treatment of diabetes mellitus.

Since that time the studies have continued with the original group and the series has been expanded to include more difficult types of unstable diabetes. With these further observations some earlier beliefs have been confirmed, others attenuated or amended, and still others greatly changed.

Present status of the study group. At this time the total series, as reported at the recent meeting of the American College of Physicians,[2] consists of 210 patients who have received biguanides, while 173 have been studied well enough to classify them in table 1. Combining Group I and Group II shows that 88 per cent had a blood-sugar lowering effect but totaling Group II and Group III indicates that 38 per cent either were not successful in lowering blood sugar in the dose used or could not tolerate the drug.

TABLE 1

Classification of 210 patients receiving biguanides

		Number	Per Cent
Group I	(Successful)	107	62
Group II	(Discontinued)	43	26
Group III	(Failure)	23	12
		173	100
Group IV	(Not in study)	37*	
		210 received biguanides	

*Includes six patients who, though free of side effects or toxicity, were dropped from Group I because placebo studies showed no active diabetes.

Presented at the Eighteenth Annual Meeting of the American Diabetes Association in San Francisco on June 22, 1958.

From the Joslin Clinic and the New England Deaconess Hospital, Boston, Massachusetts.

This study group, while neither a random nor a consecutive group, was diabetically heterogeneous because 30 per cent were juvenile-onset diabetics, 51 per cent had a known duration of diabetes of two to thirty-five years and only 19 per cent were new cases with a duration of one year or less. The age and known diabetic duration of this group are shown in figures 1 and 2. Eighty-two patients received one of the biguanide preparations for five to eighteen months. The average duration of treatment is thirteen months while fifty-four have been observed longer than one year.

PRESENT STATUS OF THE BIGUANIDES

At present there are four closely allied biguanide analogues (figure 3) which vary, but in general those with the greatest hypoglycemic effectiveness also have the most side effects. The knowledge of DBI pharmacology is incomplete. To date no means of measurement are available in vivo. A summary of the available data, particularly that of Williams and his group,[3,4,5] Ungar[6] and Wick,[7] indicates that the disappearance of glucose following DBI administration is probably due to increased anaerobic glycolysis. Although there is a difference in chemical structure between Synthalin (guanidine-decamethylene-guanidine) and the biguanides (figure 4), the metabolic properties of the two drugs show a striking resemblance in their known mode of action and clinical behavior.[2,8] However, the biguanides, unlike Synthalin, have so far not produced known toxic effects.

The hypoglycemic effect. The ability to lower blood sugar in many diabetics is one of the least debatable characteristics of the biguanides and has been well documented during the past year by Pomeranze,[9] Williams,[4] Weller,[10] Skillman,[11] Lambert[12] and others. An illustrative case (No. 166) is presented in figure 5.

Case 166. This forty-eight-year-old male, 73 in. tall, weighing 198 lb., had a known diabetes duration of eight years. His daily insulin dose was 25 units of NPH.

FIGURE 1

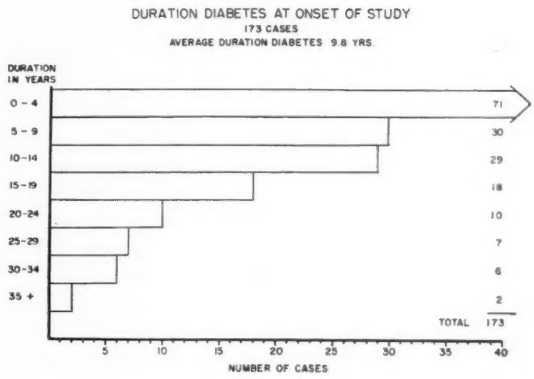
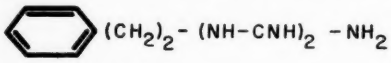


FIGURE 2

THE BIGUANIDES

(DBI) PHENETHYL-FORMAMIDINYLIMINOUREA

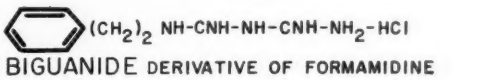


OTHER ANALOGS:

(DBB) NORMAL AMYL-FORMAMIDINYLIMINOUREA
(DBTU) ISOAMYL-FORMAMIDINYLIMINOUREA
(DBC) METHYLBENZYL-FORMAMIDINYLIMINOUREA

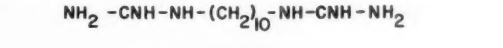
FIGURE 3

(DBI) PHENETHYL-FORMAMIDINYLIMINOUREA HCl



A BIGUANIDE DERIVATIVE OF FORMAMIDINE

(SYNTHALIN) GUANIDINE-DECA-METHYLENE-GUANIDINE



A DIGUANIDINE

FIGURE 4

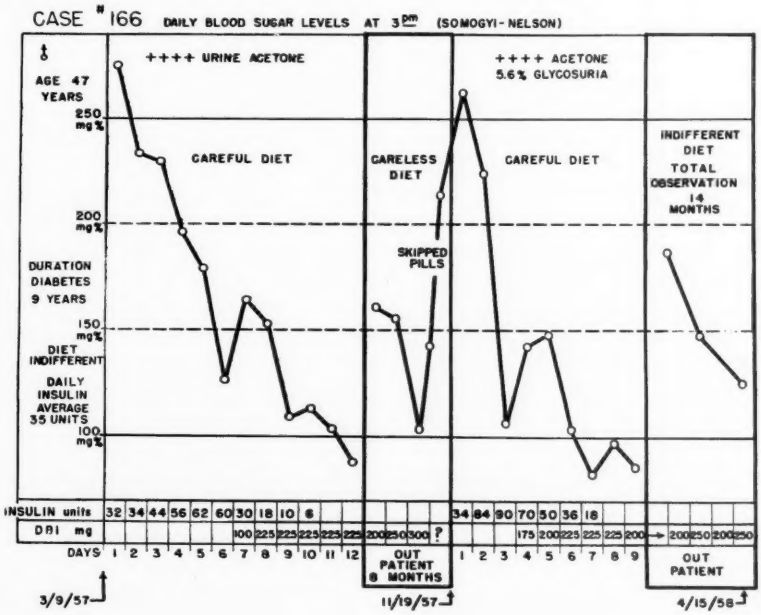

CASE # 166 DAILY BLOOD SUGAR LEVELS AT 3 PM (SOMOGYI-NELSON)
AGE 47 YEARS
DURATION DIABETES 9 YEARS
DIET INDIFFERENT
DAILY INSULIN AVERAGE 35 UNITS
++++ URINE ACETONE
++++ ACETONE 5.6% GLYCOSURIA
CAREFUL DIET
CARELESS DIET
CAREFUL DIET
INDIFFERENT DIET
TOTAL OBSERVATION 14 MONTHS
SKIPPED PILLS
250 mg%
200 mg%
150 mg%
100 mg%
INSULIN units 32 34 44 56 62 60 30 18 10 6 34 84 90 70 50 36 18
DBI mg 100 225 225 225 225 200 250 300 ? 175 200 225 225 200 200 250 200 250
DAYS 1 2 3 4 5 6 7 8 9 10 11 12 1 2 3 4 5 6 7 8 9
OUT PATIENT 8 MONTHS
OUT PATIENT
3/9/57
11/19/57
4/15/58

FIGURE 5

He was hospitalized with a diet of 161 gm. of carbohydrate, 88 of protein and 83 of fat. During this hospitalization 300 mg. of DBTU successfully replaced the insulin. During the next eight months his control was adequate with a 1,900 calorie diet and 200 mg. of DBTU daily. Marked glycosuria was noted during a placebo period. He then became careless with his diet and biguanides, omitting them and taking them only when he had "bad tests." He was found to be in early acidosis with 4 plus urine acetone, glycosuria 5.6 per cent and a fasting blood sugar of 220 mg. per cent. He was hospitalized and again regulated, though requiring as much as 96 units of insulin daily to accomplish this.

Since discharge his regulation has been adequate with no insulin, a diabetic diet and varying doses of biguanide daily. After one year there is no evidence of change in liver and renal function or hematological studies.

The side effects. The incidence of side effects is still high. Except for a more perceptive clinical acumen in the investigators reflected by better technics of administration and case selection, little has occurred in the past year to decrease the gastrointestinal side effects which as yet appear unrelated to any organic toxicity or damage. There is a narrow area between the therapeutic dose and that producing side effects in many patients.

Toxicity. Because of earlier Synthalin experience, the toxicity studies assume an increasing importance. The acute studies[13,5] in experimental animals show that death by hypoglycemia is produced in animals receiving the appropriate dose of DBI, but no gross or histologic lesions are found. In contrast to the other animal studies, those with the dog have proved unsatisfactory. Ungar[6] reports that blood sugars respond inconsistently and death occurs in a few days, often with a normal blood sugar but with elevated serum phosphorus and low serum calcium. These atypical dog experiments appear to parallel studies with Paludrine.[14]

In our own series of diabetic patients there have been no significant changes in the liver function studies which included bromsulfalein retention, alkaline phosphatase, cephalin flocculation, thymol turbidity and blood bilirubin. Hematological studies, including hemoglobin determination, white blood count and blood differential counts, were consistently normal while renal function studies, including nonprotein nitrogen and urinalysis, showed no abnormal variation. It is significant that this lack of change occurred even in some of our patients who had kidney damage secondary to nephropathy and abnormal liver function values due to liver disease prior to biguanide administration.

These findings, as well as normal serum transaminase levels, have also been reported by the previously mentioned investigators.

STUDIES WITH JUVENILE DIABETES

Attempts to alter the course and outcome of juvenile diabetes or to stabilize the action of insulin appear justifiable because of the present natural course of diabetes in childhood. After an acute onset, often in severe ketoacidosis, and after a temporary remission phase, intensification of diabetes occurs. The remission phase is recognized in one out of three childhood cases and usually occurs in the third month after treatment, generally lasting another three to twelve months. Gradually diabetes intensifies to a total disease. By the fifth year of duration some 95 per cent manifest this total state, at which time the insulin requirement of our patients has risen to an average of 0.5 units per pound of body weight. Perfect chemical control of diabetes with present forms of insulin at this stage is difficult. Overcorrection produces reactions, distressing to patients and their families, who often follow programs producing chronic states of hypoinsulinism.

In the course of their diabetic lives, these patients are exposed to three potentially lethal complications: coma, sepsis and vascular disease, with the last becoming the main problem in long-term juvenile-onset patients and its prevention the main objective. Although a debated point, it seems logical to think that if excellent control can be achieved without distressing hypoglycemia, better results may follow.

Material. In the past eighteen months (Jan. 1, 1957, to June 1, 1958) 100 patients with growth-onset of diabetes (under eighteen years old at recognition) have been treated with one of the biguanide drugs. From the original 100, 18 were excluded from the study because they received doses of 50 mg. daily (a subhypoglycemic dose) in a separate study by Dr. Jørn Ditzel, who attempted to restore a normal tone to the abnormally dilated vessels demonstrable in the bulbar conjunctiva. Ten others were excluded: for lack of conclusive data, six; unco-operative patient, three; no proof of active diabetes, one. Seventy-two patients remained for therapeutic evaluation.

Patient selection. The selection of patients of recent onset for this study was often but not exclusively on the basis of family experience with diabetes and insulin, such as the second sibling to develop diabetes or the offspring of a diabetic parent. Otherwise, for the

most part, patients were transferred after several weeks of insulin therapy to ensure their understanding of its use for emergencies. Long-term cases were chosen because of challenging problems, primarily for unusually severe reactions, which were often not accompanied by warning symptoms and led to disasters such as automobile accidents. Other patients were unusually labile or had long-continued lipodystrophy. Except for some recent onset cases, juvenile patients whose progress was completely satisfactory with insulin were usually not included in this study.

Clinical characteristics. At the onset of the study the range for duration of diabetes was 0.1 to 35 years, ages 4 to 46 years and daily insulin dose 4 to 100 units. Thus, the average duration was nine years, age sixteen and a half years and daily dose of insulin 40 units.

Routine of study. The management of these patients was conducted usually during hospitalization, occasionally while on outpatient observation. All were treated with the dietary regulation employed in all Joslin Clinic patients. In the hospital blood determinations for true glucose were made fasting, at 11 a.m. and at 3 p.m. Daily twenty-four hour quantitative tests for urine glucose were made as well as before-meal and bedtime qualitative tests. The liver and kidney function and hematological studies previously described were continued in many of this group. Outpatients reported frequently for blood and urine analysis and kept records of qualitative tests, reactions, complications, diet and weight.

Extensive use of placebos was made in this study. When control was established, a placebo was substituted for the DBI until rising blood sugars removed all doubt of the activity of the diabetes. If no evidence of active diabetes could be found, the patient was removed from the study series until such proof was found.

Results. According to the criteria in tables 2 and 3, the results are as follows:

	Number	Per cent
Group I —(Successful)	35	47
Group II —(Discontinued)	29	42
Group III—(Failures)	8	11
	72	100

Combining Groups I and II indicates that sixty-four (89 per cent) had significant blood-sugar lowering while the addition of Groups II and III shows that thirty-seven (53 per cent) discontinued biguanide therapy because of side effects or failure in the doses used.

Of the thirty-five in Group I, thirty still continue on biguanides, fourteen without insulin and sixteen with supplemental but smaller doses of insulin. This group

TABLE 2

Classification of cases according to the blood-sugar lowering response to the biguanides

Group I	SUCCESSFUL. A demonstrable blood-sugar lowering effect according to criteria in Table 3.
Group II	DISCONTINUED. This group had a demonstrable blood-sugar lowering effect but were unable to tolerate the drug. Discontinued from the series because of side effects.
Group III	FAILURE. No demonstrable blood-sugar lowering effect in the doses administered.
Group IV	NOT IN STUDY. This group includes uncooperative patients, inconclusive results, those under observation with minute doses as part of another study and certain patients who could not demonstrate diabetic activity during the placebo phase of the study. These are not included in results unless they had side effects.

TABLE 3

Criteria for "blood-sugar lowering effect"

1. All patients were on a prescribed diabetic diet prior to and during the evaluation period. Those on an outpatient status continued this diet.
2. Three types of patients were studied:
   (a) those receiving no previous insulin had to show a consistent drop of at least 25 per cent in average blood sugar level while receiving the biguanides. This had to be below the level of 150 mg. per cent venous Somogyi-Nelson blood sugar values on specimens taken at 11 a.m. and 3 p.m.
   (b) those previously maintained with insulin, who were able to replace this with one of the biguanides, had to maintain blood sugar levels as good or better than they had with insulin.
   (c) those who were unable to replace their insulin fully with the biguanides had to maintain their status with an insulin dose less than 50 per cent of the original plus the biguanide.
3. Proof of active diabetes had to be established in each case by either:
   (a) a diabetic blood sugar level (postprandial Somogyi-Nelson venous value of 150 mg. per cent or higher) prior to the trial period, and
   (b) a return to diabetic blood sugar levels when DBI was discontinued or a placebo substituted for the biguanide in spite of a constant diet.

of thirty has been observed from one to sixteen months (average seven months). Those on combined therapy average 152 mg. of DBI in daily divided doses plus an average of 10 units of insulin (range 4 to 40 units daily). Those on DBI alone average 110 mg. daily. Figure 6 shows the duration of biguanide treatment in both groups related to the duration of diabetes. It is evident that the shortest duration cases show the best results with DBI alone. This is also indicated in table 4.

In these patients concurrent infections, pyelonephritis (2), rubella (1), abscessed tooth (1), upper respiratory

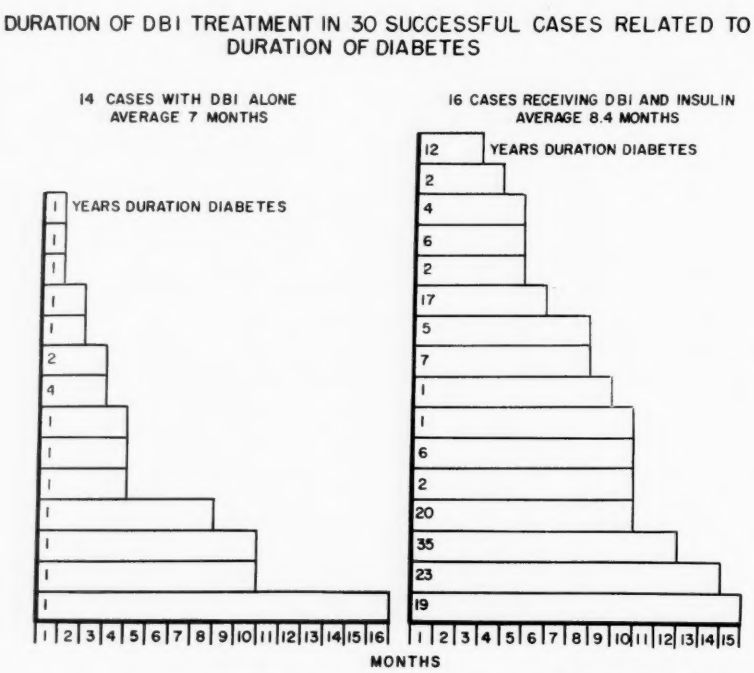


FIGURE 6

TABLE 4

Comparative characteristics of the study group

	Average Age at Onset of Study	Average Duration of Diabetes at Onset of Study	Average Insulin Dose at Onset of Study
The study group (seventy-two cases)	16.5 yr. (4-46 yr.)	9 yr. (0-35 yr.)	40 units (4-100 units)
"Successful" Group I cases (thirty-five cases)	15 yr. (4-46 yr.)	6.6 yr. (0-35 yr.)	31 units (4-100 units)
Remaining on DBI (thirty cases)	14 yr. (4-46 yr.)	6 yr. (0-35 yr.)	28 units (4-100 units)
Remaining on DBI alone (fourteen cases)	10 yr. (4-17 yr.)	1.3 yr. (1-2 yr.)	18 units (4-40 units)

infections, were followed by hyperglycemia and/or glycosuria, which was also present following accidental or intentional omission of drug therapy as well as gross dietary indiscretions. These were often corrected by increase in biguanide dosage. Well over 75 per cent of the blood sugar tests are maintained at normal levels after hospitalization and during outpatient follow-up. The expected average "true insulin requirement" dose of 0.5 units per pound of body weight is far in excess of the 10 units daily average. The average dose in those still requiring insulin was reduced by 64 per cent.

Case histories. No. 48 (figure 7) is a thirteen-year-old girl with diabetes of recent onset. Her blood sugar became stabilized with DBI, 200 mg. daily, plus diet. These values became elevated when a placebo was substituted for the biguanide, while the patient was still following a weighed diet, and returned to normal when the oral medication was resumed. After several months of outpatient observation, the biguanide was discontinued for several days. During this period she ate a weighed diet but hyperglycemia and glycosuria were again found. These returned to normal levels with a resumption of the DBI. She has been observed for sixteen months, has maintained good diabetic control and well-being as well as proper growth and development.

Comment: It should be noted that although this juvenile diabetic is not in remission and has been well regulated with DBI, she did not show urine acetone during the placebo period while both hyperglycemia and glycosuria were present.

Case 88 (figure 8) is a more severe diabetic whose diabetes was present for twenty of his thirty-two years of age. His insulin dose averaged 80 units or more daily

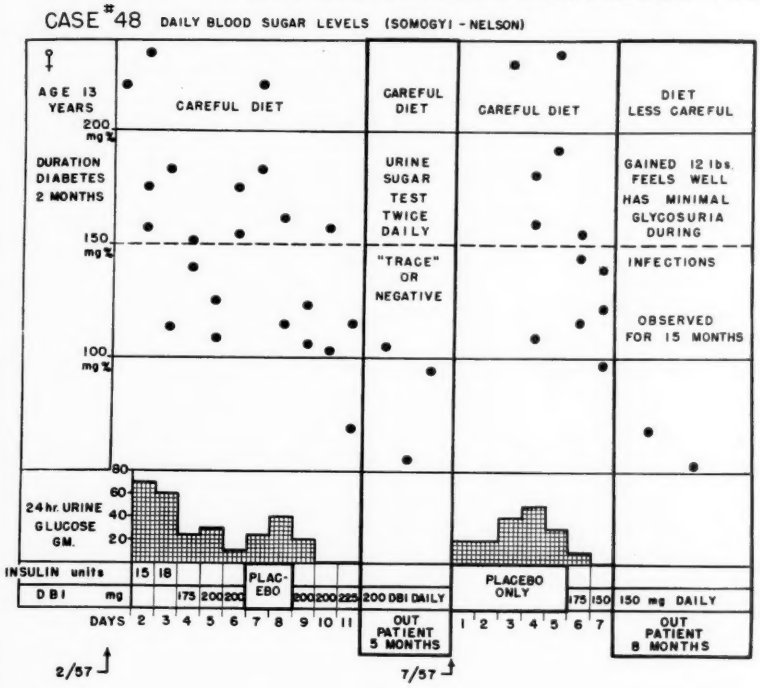


FIGURE 7

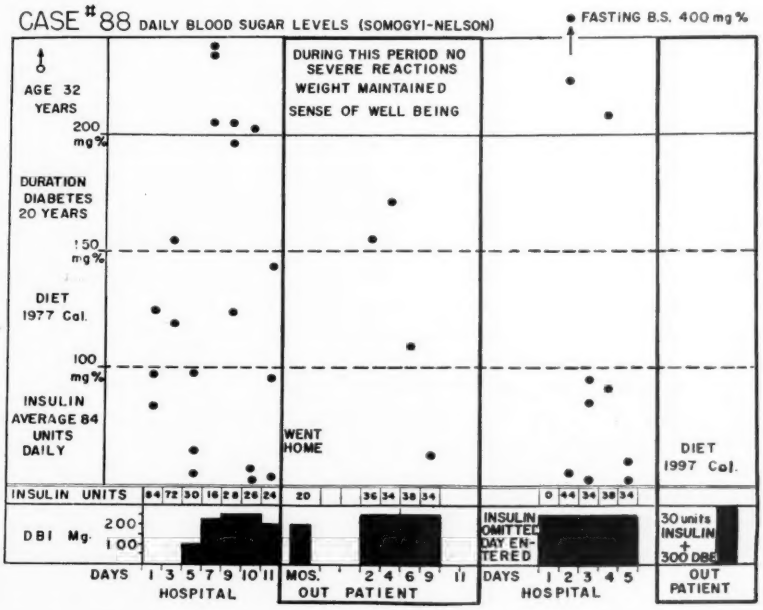

CASE #88 DAILY BLOOD SUGAR LEVELS (SOMOGYI-NELSON)
FASTING B.S. 400 mg%
AGE 32 YEARS
DURING THIS PERIOD NO SEVERE REACTIONS WEIGHT MAINTAINED SENSE OF WELL BEING
200 mg%
DURATION DIABETES 20 YEARS
150 mg%
DIET 1977 Cal.
100 mg%
INSULIN AVERAGE 84 UNITS DAILY
WENT HOME
DIET 1997 Cal.
INSULIN UNITS
84 72 30 16 28 26 24
20
36 34 38 34
0 44 34 38 34
DBI Mg. 200 100
INSULIN OMITTED DAY ENTERED
30 units INSULIN + 300 DBI
DAYS 1 3 5 7 9 10 11
HOSPITAL
MOS. 2 4 6 9 11
OUT PATIENT
DAYS 1 2 3 4 5
HOSPITAL
OUT PATIENT

FIGURE 8

and his blood sugars varied with many severe reactions. He tolerated the biguanides nicely and after fifteen months of combined insulin and DBI therapy, his weight has remained stationary, there is no evidence of toxicity and he has been free from severe reactions.

Acidosis. Although acetonuria was sometimes seen, no one developed ketosis or coma (CO_2 of 9 mEq. or below). This was partly due to careful observation and the

prompt administration of insulin if needed. On the other hand, several patients with extremely high blood sugar levels and glycosuria who had been receiving DBI returned to normoglycemic levels during this period with very small doses of insulin, lending credence to the observation that DBI may reinforce or augment insulin activity. No attempt was made to treat any patient in severe acidosis or coma with the biguanides.

Insulin reactions. The absence of severe hypoglycemic reactions was striking. No convulsions, loss of consciousness or severe confusion were seen in this series, even in those patients with combined DBI and insulin therapy. After initial stabilization blood glucose levels below 60 mg. per cent were rarely observed. However, one five-year-old child receiving biguanide alone had a blood sugar level of 25 mg. per cent during the first week of trial and remained conscious, while an adult had hypoglycemic levels of 18 and 23 mg. per cent without severe reaction symptoms.

Growth. Physiological control paralleled clinical control. Linear growth was satisfactory in all cases. In the fourteen maintained on DBI alone the average increment in height was 0.4 in. for two months, 0.8 in. for six months, 1.5 in. for twelve months. The average gain in weight was 3.5 lb. for four months and 9.5 for twelve months.

Partial remissions. All fourteen patients maintained with DBI alone and who formerly required an average dose of 18 units have continued without requiring insulin for periods of time greater than expected. Three comparable groups have been studied by one of us (P. W.) recently. Twenty-five juvenile diabetics with diabetes of recent onset were treated with insulin and sulfonylurea. None was able to discontinue insulin or even reduce it, and they followed the natural course of juvenile diabetes, eventually requiring 0.5 unit of insulin per pound. In like fashion, no long-term juveniles replaced insulin in part with sulfonylureas, although these, of course, were not in the comparable early onset group. Another group of juveniles early at the onset of their diabetes received subdiabetogenic doses of synthroid and another group received subdiabetogenic doses of cortisone in an attempt to induce and prolong remission by reproducing islet hypertrophy and hyperplasia as shown in experimental diabetes. These last two groups of fifty patients with one exception were unable to discontinue insulin and, with this exception, have followed the natural course of juvenile diabetes, requiring ever increasing doses of insulin up to 0.5 unit per pound of body weight.

The expected number of remissions in our juvenile cases is one in three of these DBI study patients. Yet all fourteen continue with the drug, although the critical three- to five-year period for onset of total diabetes has not yet been reached.

Vascular lesions. Although the length of observation certainly does not warrant predictions regarding the structural changes in small blood vessels, physiological responses at the microscopic level have been observed by Dr. Jørn Ditzel. He has reported[15,16] pathological changes, i.e., dilatation of bulbar conjunctival venules associated with arteriolar narrowing, capillary irregularity and intravascular sludging. DBI had no effect on arteriolar constriction but alone or in combination with insulin seemed to have favorable effect on venular dilatation.

Other complications. In the short period of study no complicating sepsis or neuropathies have occurred.

Toxicity. No toxicity has been shown in liver function tests including bromsulfalein retention, cephalin flocculation, thymol turbidity, alkaline phosphatase, NPN, white and red cell counts, hemoglobin, differential counts and complete urine analyses.

Side effects. Side effects, however, were numerous. These include loss of appetite, thirty-nine; nausea, twenty-nine; vomiting, twelve; diarrhea, three; lassitude, three. These effects appear most commonly twenty-four to forty-eight hours after the inception of treatment and when the drug is omitted, usually subside in twenty-four hours.

Technics of administration. In severe growth-onset diabetes, the administration of biguanides can be best accomplished by attempting to substitute DBI combined with insulin after the patient has achieved maximal control with insulin. The insulin is gradually decreased while the DBI is increased until the maximal tolerated DBI and the lowest possible insulin dose consistent with good regulation are reached. If side effects ensue, the biguanide dose is lowered or omitted. Past training with insulin by the patient or his family minimizes chances for errors in management leading to ketoacidosis. Immediate resumption of the pretherapy dose of insulin is advised for emergencies requiring omission of the day's total DBI. This may be supplemented with regular insulin by test. If emergencies arise after the absorption of biguanide, not more than one half of the usual supplementary insulin is ordinarily necessary. The incidence and severity of side effects are lessened by a slow transfer to the drug. Often they are less frequent in patients treated on an outpatient basis because of their slower transfer in comparison with hospitalized cases.

The biguanides are not as facile or versatile as insulin. The activity appears to start several hours after ingestion and in its present form usually disappears in six to eight hours. This makes multiple doses necessary and makes the control of the really severe diabetic almost impossible without supplemental insulin overnight. Because the intensity of degree and the speed of development of hypoglycemia are less than those of insulin, the severity of hypoglycemic reactions is minimized, but this time-action curve makes it unsuitable for emergencies. A total daily dose of over 150-200 mg. is rarely well tolerated in juveniles.

DISCUSSION

The biguanides are enigmatic compounds which are discouraging because of the high incidence of side effects and rewarding when an otherwise unstable, fluctuating diabetic with almost incapacitating reactions becomes better regulated with a decreased dose of insulin plus the DBI.

"Many are called but few are chosen" aptly describes our experience with this study group whose statistical attrition is illustrated in figure 9.

In addition to the gastrointestinal side effects, the capriciousness of DBI is underlined by the fact that an occasional patient (case 49) is well regulated for long periods with normoglycemia, no glycosuria, without weight loss and yet feels tired with no sense of well-being. Another patient (case 56) was well regulated for a number of months and then gradually showed elevated blood sugar levels without any discernible cause. One patient (case 121) failed with DBB but had a good response to phenethylbiguanide, while another (case 259) had no effective hypoglycemia with DBI but was successful with the less active n-amyl analogue.

A serious problem is the inability to give a dose of DBI large enough to be effectively hypoglycemic in many patients. Except in cases of insulin resistance, this is not true with insulin which can be administered in doses sufficient to lower blood sugar levels even though this may create reaction problems. The sulfonylureas, when effective in carefully chosen patients, often accomplish this with a singularly simple and stable dose, but with the present biguanides the dose required varies according to the severity of the diabetes and yet the narrow therapeutic range makes it sometimes difficult to give an effective dose that can be tolerated.

Some clinical resemblance to Synthalin A and B is striking, but from the standpoint of toxicity, there appears to be no similarity. Comparison of biguanides and Synthalin inevitably brings to mind the experience with tolbutamide and carbutamide, the sulfonylureas which show much resemblance until variations in toxicity are considered. The wide margin of safety of Paludrine indicates that biguanide per se is not inherently toxic at therapeutic levels.

However, increasing numbers of patients with growth-onset type of diabetes and its attendant lability are faced with rapid rise and fall of blood sugar level, which often defies the most skillful attempts at control. In these patients, the combined biguanide and insulin therapy, when tolerated, smoothes the blood sugar pat-

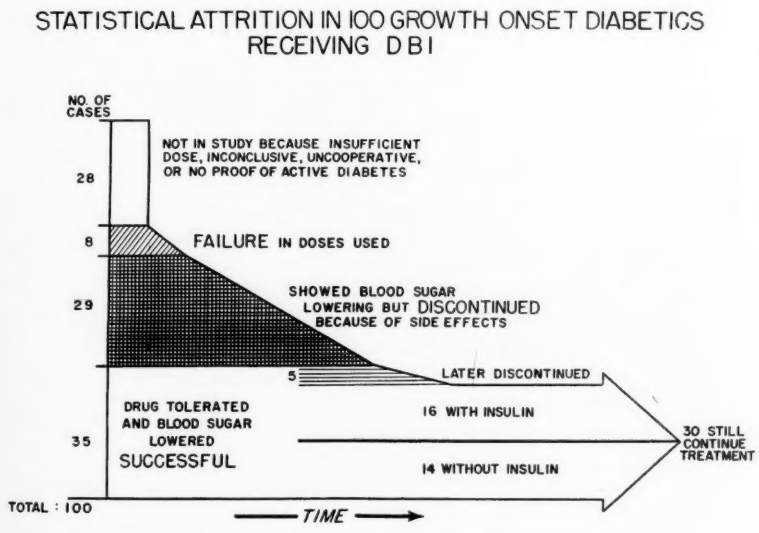


FIGURE 9

tern and greatly reduces severe insulin reactions, frequently resulting in blood sugar regulation better than that obtained with insulin alone.

The short-term cases treated with biguanide alone have done better than expected. While their eventual need for insulin may not be revealed until the third to fifth year of their diabetes, at present they have done better than comparable study groups. The eventual effect upon the natural course of their diabetes cannot be determined at the present time.

Although the site of action has not been clarified, the clinical effect appears to be that of reinforcement or potentiation of exogenous or endogenous insulin.

Disturbing side effects of unknown mechanism limit the use of this drug but experience gained with the passage of time has lessened their frequency and intensity. The experience with the side effects from the biguanides has not been much more disturbing than were the nocturnal hypoglycemic reactions produced in the early trials with Protamine Zinc insulin.

SUMMARY

1. Seventy-two growth-onset cases of diabetes, receiving a regulated diet, were treated from one to sixteen months with the biguanides DBI and DBB and in some cases a smaller supplemental dose of insulin. Eighty-nine per cent responded with significant blood sugar lowering, but 53 per cent of the total group discontinued the drug because of side effects or failure to lower blood sugar levels in the doses used.

2. Thirty cases still continue treatment for one to sixteen months (average seven months). Fourteen of these are being treated with DBI and diet alone, while sixteen others also receive significantly lowered doses of insulin.

3. Gastrointestinal side effects occurred in almost one half of the patients although these were not always severe enough to discontinue treatment.

4. No clinical evidence of toxicity occurred and none by tests of hepatic, renal or hematologic function.

5. Significant severe hypoglycemic reactions were not found.

6. Linear growth and gain in weight followed the expected normal curves.

7. Restoration of normal venular tone in the abnormally dilated venules of the bulbar conjunctiva was observed in some cases.

8. Short-term cases treated with biguanide alone appear to have done better than expected in delaying natural intensification of diabetes and the long-term cases did better than expected in respect to insulin requirement.

9. An attempt at stabilization is justifiable in the usually labile, severe growth-onset diabetics. When side effects can be eliminated or the biguanides tolerated, DBI and DBB appear to be useful adjuncts in the total therapy of juvenile diabetes.

SUMMARIO IN INTERLINGUA

Le Uso Clinic Del Biguanidos E Lor Rolo In Le Stabilisation De Diabete De Typo Juvenil

1. Septanta-duo diabeticos de etate de crescentia, recipiente dietas regulate, esseva tractate durante periodos de inter un e dece-sex menses con le biguanidos DBI e DBB, supplementate—in certe casos—per doses reducite de insulina. Octanta-nove pro cento reageva per significative reductiones del nivellos del sucro sanguinee, sed 53 pro cento del gruppo total interrumpeva le uso del droga a causa de su effectos lateral o proque—in le doses usate—illo non reduceva le nivello del sucro de sanguine.

2. Al tempore presente, trenta del patientes continua le tractamento, initiate inter un e dece-sex menses retro (con septe menses como duration medie). Dece-quatro del trenta es tractate con DBI e dieta sol, durante que le remanente dece-sex recipe etiam significativemente reducite doses de insulina.

3. Effectos lateral gastrointestinal occurreva in quasi un medietate del patientes, sed illos non esseva satis sever in omne le casos pro requirer le discontinuation del tractamento.

4. Occurreva nulle toxicitate secundo le evidentia clinic e nulle secundo tests del function hepatic, renal, o hematologic.

5. Reactiones hypoglycemic de grado significativemente sever non esseva trovate.

6. Le crescentia linear e le ganio de peso sequeva le expectate curvas normal.

7. In certe casos, esseva observate le restauration de un normal tono venular in le anormalmente dilatate venulas del conjunctiva bulbar.

8. Casos a curte durantia tractate con biguanido sol beneficiava a grados inexpectate quanto al retardo del intensification natural de diabete, e le casos a longe durantia beneficiava a grados inexpectate con respecto a lor requirimentos de insulina.

9. Le effortio de effectuar un stabilisation es justificate in le usualmente labile casos sever de diabete al etate de crescentia. Quando le effectos lateral pote esser eliminate o quando le biguanidos es ben tolerate, DBI e DBB pare esser utile adjunctos in le therapia total de diabeticos juvenil.

ACKNOWLEDGMENT

Appreciation is expressed to the U. S. Vitamin Corporation for assistance and for the biguanide compounds used.

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Changes in the ground substance accompanying experimental xanthomatosis in the rabbit were also studied by Wang, Strauss and Adlersberg (*Arch. Path. 64*:501, 1957). The original observation of a bluish violet hue of the ground substance in sections stained with hematoxylin-eosin led to further studies of the relationship between "acid mucopolysaccharides" of the ground substance as demonstrated by staining with colloidal iron, and the deposition of lipids during cholesterol feeding of rabbits. A group of 120 "chinchilla gray" male rabbits was fed cholesterol-treated chow from two weeks to ten months, receiving approximately 1 gm. of cholesterol per day. Of this group, fifty-two were selected for the study of experimental atherosclerosis and lipoidosis after varying periods of cholesterol feeding. Tissues were fixed in 10 per cent formalin and sections were stained with hematoxylin-eosin.

Although there is some question about the specificity of the procedure, colloidal iron staining was interpreted to indicate the acid mucopolysaccharides of the ground substance and gave a bright turquoise color. For reference, umbilical cord was stained by the same technic. The skin of untreated control rabbits showed no foam cells and only small quantities of the colloidal iron positive material in the ground substance of the connective tissue surrounding the hair follicles of the dermis. Animals fed cholesterol showed the early appearance of the blue material coinciding with the appearance of foam cells followed by a decrease in "mucopolysaccharides" as foam cells increased. Lipids seemed to be deposited initially in a fine matrix of this material, both within and outside the cells.

The cartilaginous tissue matrix of the joints of untreated young animals was characterized by a bright blue color with the colloidal iron stain. Cholesterol feeding was accompanied by the usual deposition of foam cells in the synovial membrane, periosteum, bone marrow and lacunae. A moderate increase in "mucopolysaccharides" was seen in the connective tissue around the arteries, in the periosteum and in the bone marrow along the trabeculae. The cytoplasm of the foam cells was stained pale blue. The difference in staining observed in different normal tissues either indicated a quantitative variation in "acid-mucopolysaccharide" concentration, or reflected a changing color reaction due to the fact that the type of chondroitin sulfate varied from tissue to tissue.

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The Hexosemonophosphate Shunt and Adaptive Hyperlipogenesis

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INTRODUCTION

A recent study of the effect of antecedent food intake pattern on hepatic lipogenesis (Tepperman and Tepperman)[1] revealed an extraordinary range of lipogenic activity of surviving liver slices derived from rats and mice which had been prepared in a number of ways. In confirmation of the work of others,[2] the incorporation of the C^{14} label of acetate into the lipid fraction was found to be negligible when the slices were obtained from starved rats. When rats were trained to eat their entire day's ration in one hour ("trained") their liver slices showed comparatively "supernormal" lipogenesis. Similarly, liver slices of aurothioglucose obese mice which had been overeating for several months incorporated much more of the precursor label into fat than did slices of lean fed controls (see also Mayer et al.).[3]

When rats were starved for forty-eight hours and refed a nutritionally complete high carbohydrate, low fat diet for twenty-four hours their liver slices exhibited strikingly high lipogenic activity when tested under the same conditions employed for the experiments described above.[1,4] We have called the "supernormal" lipogenesis of overnutrition, "training" and refeeding "adaptive hyperlipogenesis" because the lipogenic activity of the liver in each circumstance seems to us to be teleologically appropriate to the nutritional state of the animal.

The refeeding phenomenon appeared to be particularly useful for exploring the mechanisms involved in adaptive hyperlipogenesis, especially since a correlation plot of lipogenic activity versus initial glycogen level of the liver (figure 1) revealed that there is a good correlation (coefficient 0.84) between these parameters for many groups of control, fasted and "trained" rats, but that two refed groups were far outside the 3x S.E. of the Estimate boundary which presumably includes 99 per cent of the observations in a population. This suggested strongly that the slices from refed rats developed a lipogenic advantage over and above that afforded by their very high initial glycogen content.

The demonstration by Shaw, Dituri and Gurin[5] that the reaction crotonyl CoA→butyryl CoA is a locus of the defect in lipogenesis in diabetes and Langdon's[6] demonstration that there is a specific requirement for TPNH at this step suggested that this critically placed reaction might be a locus of "training" in those circumstances in which relatively "supernormal" lipogenesis occurs. The demonstration (Tepperman and Tepperman)[1] that most of the lipogenic advantage of the liver slices from "refed" rats disappears when one compares lipogenesis in nicotinamide-fortified whole homogenates of normal and "refed" livers emphasized the probability that variations in lipogenesis are more likely to be related to fluctuating supplies of critically needed cofactors than to variations in activity of the apoenzymes involved in the synthetic reaction. Accordingly, refed animals were prepared and their livers were assayed for TPN reducing activity of the hexosemonophosphate shunt dehydrogenases by the method of Glock and McLean.[7] (See Materials and Methods.) When the rats were sacrificed twenty-four hours after the beginning of refeeding (which oc-

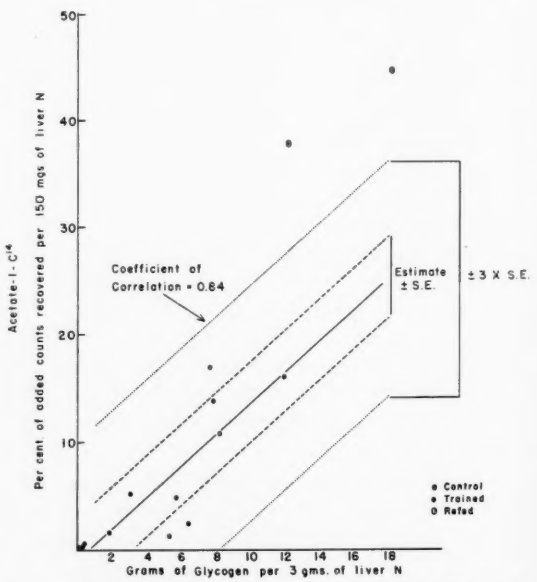

FIG. 1. Correlation of initial glycogen level and lipogenesis performance of liver slices. Data of Tepperman and Tepperman.[1]

Presented at a meeting of the American Physiological Society in Philadelphia on April 16, 1958.

From the Department of Pharmacology, State University of New York Upstate Medical Center, Syracuse, New York.

curred at the end of a forty-eight-hour fast) the enzyme activity was 300-400 per cent of the level found in fed controls, depending on the reference standard used for the calculation. Since these livers had a history of having had negligible lipogenic activity and nearly normal shunt activity only twenty-four hours before, the refeeding phenomenon seemed to us to offer an extraordinary opportunity to observe the change in lipogenic capacity over virtually its entire spectrum in a very short time, and to make observations on the concurrent reorganization of the cell's biochemical machinery which might be related to the process under study. The results of the following experiments, then, constitute a sort of moving picture of certain changes in chemical morphology and enzyme activity that occur during the first forty-eight hours of refeeding. They are presented in support of the view that a fluctuating supply of TPNH is one important determinant of lipogenic activity in liver.

MATERIALS AND METHODS

Adult male albino rats (Albino Farms) were used. Their mean weight at the beginning of the forty-eight-hour period of starvation was approximately 300 gm. A high carbohydrate, low fat, 21 per cent protein diet[8] was fed ad libitum during the refeeding period. Six rats were killed at the end of the forty-eight hour fast and 3, 6, 12, 24 and 48 hours after the beginning of refeeding, measurements were made of body weight, total liver weight, liver nitrogen, liver glycogen, liver total fat in some experiments, incorporation of acetate-1-C^{14} label into total fat and hexosemonophosphate shunt dehydrogenase activity by methods previously used in this laboratory.[1] At least one chow-fed control was studied each time an experiment was done. It should be emphasized that the liver measurements were made on aliquots of the same livers. The shunt enzyme measurement included the total reduction of TPN by both glucose-6-phosphate dehydrogenase and 6 phosphogluconate dehydrogenase at *p*H 7.6. A unit of enzyme activity is defined as the amount of enzyme which reduces 0.01 micromols of TPN per minute at 20° C. and *p*H 7.6 based on readings over the first five minutes. In all cases the mean values for six rats ± S.E. are given. Probabilities are estimated by Student's t test.

RESULTS

The numerical results are given in table 1. These data are presented as absolute values and include means and standard errors for each group. In every instance in which findings are related to body weight, the prestarvation weight was used. Figures 2 to 5 represent graphic summaries of some of the information presented in table 1 recalculated on the basis of percentage of control value.

The changes in body weight, liver weight and liver nitrogen are shown in figure 2. One can see that at the end of the antecedent forty-eight-hour fast all of these parameters were low as compared with the fed controls. The most striking fluctuation was shown by the liver weight, even when it is related to the weight of the rat. It is because of the variations shown in this figure that calculations in the next two figures are based on three different reference standards.

The recovery of lipogenesis and its "overshoot" above the level seen in that of chow-fed controls is well demonstrated in figure 3. By three hours there was significant recovery; by six hours, the slices had practically half the activity of the controls. At twelve hours, three times the control amount of acetate label appeared in the lipid at the end of three-hour incubation period. There was no increase in recovery of the acetate label between twenty-four and forty-eight hours in this experiment, in which 2 μM of acetate was added per flask and, in some experiments, 50 to 70 per cent of the added counts were recovered in the lipid fraction.

The changes in shunt dehydrogenase activity on refeeding are shown in figure 4. The significant elevation at twenty-four hours is confirmed, but this is seen to be only the beginning of a much more impressive rise.

The interrelations of lipogenic activity, shunt activity and initial glycogen content of the liver are well illustrated in figure 5. Here, one can see that in the early hours of refeeding, before any appreciable change in shunt enzyme activity has occurred, lipogenic activity is beautifully proportional to initial glycogen level. At twelve hours shunt activity is 135 per cent of the fed control level and lipogenesis is much higher than one would predict from a projection of the first three points. At twenty-four hours, glycogen content is extremely high, shunt activity is more than three times normal and lipogenesis is very rapid. At forty-eight hours glycogen is reduced, but lipogenesis persists at a high level. However, shunt enzyme activity is enormous. This, then, represents a clear dissociation of initial glycogen level and lipogenic activity, since a high rate of lipogenesis is seen in the face of a markedly diminished initial glycogen level.

Since more than half of the added acetate counts appeared in the lipid fraction in many experiments at twenty-four and forty-eight hours, it seemed probable that the 2 μM addition of acetate was limiting at high lipogenesis rates. Accordingly an experiment was done in which the same number of acetate counts was added in a total of 50 μM of acetate. The results are shown in

TABLE 1

Group	Body Weight, Gm. Initial	Body Weight, Gm. Final	Liver Weight Gm./100 Gm. Body Weight	Liver Nitrogen Per Cent Wet Weight of Liver	Liver Glycogen Per Cent Wet Weight of Liver	Liver "Lipogenesis" Per Cent Added Counts Recovered Per 15 Mg. Liver N	Liver "Lipogenesis" Mols Added Acetate Recovered Per 100 Gm. Rat	Liver HMP Shunt Dehydrogenase Activity Units Per Mg. Liver N	Liver HMP Shunt Dehydrogenase Activity Units Per 100 Gm. Rat
Control	296 ± 2.6	307 ± 10.3	3.7 ± .10	3.25 ± .05	5.8 ± .24	8.1 ± 1.09	1.4 ± .19	2.1 ± .16	250 ± 24.7
48 hr. fast	292 ± 9.4	263 ± 8.7	2.2 ± .06	3.68 ± .04	1.0 ± .33	0.3 ± .06	0.03 ± .0002	1.7 ± .16	144 ± 15.5
3 hr. refed	304 ± 9.3	283 ± 7.7	2.7 ± .04	3.48 ± .06	4.1 ± .13	1.4 ± .06	0.2 ± .02	1.8 ± .26	168 ± 31.2
6 hr. refed	305 ± 5.9	278 ± 3.1	2.7 ± .14	3.10 ± .05	6.7 ± .49	3.9 ± .68	0.5 ± .08	1.8 ± .16	153 ± 19.6
12 hr. refed	302 ± 4.6	286 ± 6.1	3.7 ± .12	2.45 ± .10	13.1 ± .46	25.6 ± 2.59	3.3 ± .37	2.8 ± .64	253 ± 59.5
24 hr. refed	305 ± 7.8	290 ± 6.0	4.6 ± .17	2.15 ± .11	14.6 ± .55	65.4 ± 4.71	9.3 ± .51	7.4 ± .99	720 ± 88.4
48 hr. refed	327 ± 3.4	315 ± 2.2	4.5 ± .22	2.45 ± .11	8.5 ± .75	65.5 ± 9.95	10.2 ± 1.37	28.9 ± 4.76	3,092 ± 635.6

Note: Each figure represents the mean of determinations on six rats ± S.E. of the mean.

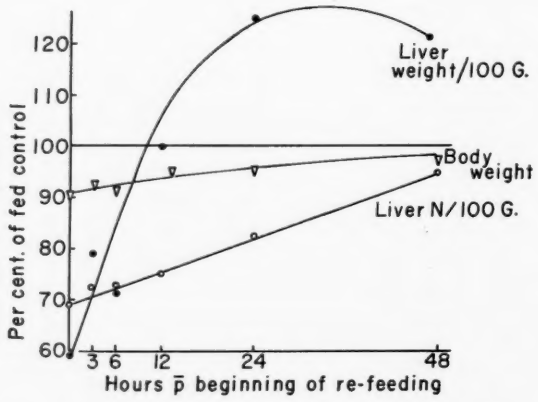

FIG. 2. Body weight, liver weight and liver nitrogen during refeeding. "/100 G" refers to initial body weight. Calculated from table 1.

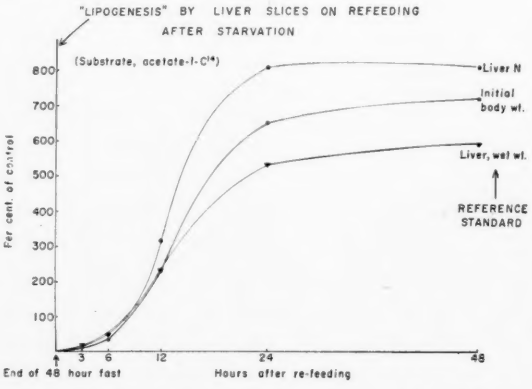

FIG. 3. Experiments done with two micromols of acetate per beaker. Lipogenesis referred to three different reference standards and calculated from data in table 1 as percentage of fed control.

figure 6. As before, there was a marked drop in initial glycogen level between twenty-four and forty-eight hours, but this time there was an *increase* in lipogenesis at the 5 per cent level of confidence. The apparently higher shunt activity at forty-eight hours in this experiment as compared with that shown in figure 4 is simply due to the fact that the control values for this experiment were slightly lower than were those for the previous one.

It was pertinent to inquire whether or not the rate of incorporation of the acetate label into the cholesterol fraction paralleled that into fatty acids. Accordingly, an experiment was done in which the incorporation into total lipid and into the digitonin-precipitable fraction was measured separately. The results, shown in figure 7, indicate that the counts recovered in the digitonide fraction of the control represented only a very small proportion of those recovered in the total lipid, and that, although the refeeding maneuver sharply increased incorporation in total lipid, it resulted in a significantly diminished incorporation in the digitonide. Thus at forty-eight hours after refeeding only 0.03 per cent of the counts recovered in the total lipid were in the digitonin-

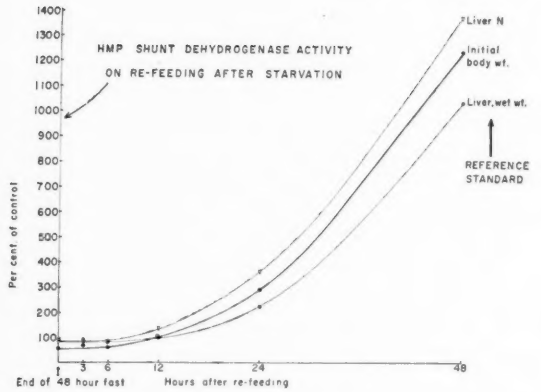

FIG. 4. Data are calculated from table I as percentage of fed control.

precipitable fraction.

The high lipogenic activity of the slices from refed rats suggested the possibility that a net increase in total liver fat might be observed during the refeeding period. Figure 8 shows a significant increase in liver fat from the twenty-fourth to the forty-eighth hour of refeeding.

The finding of a high HMP shunt dehydrogenase activity at forty-eight hours was the basis of an experi-

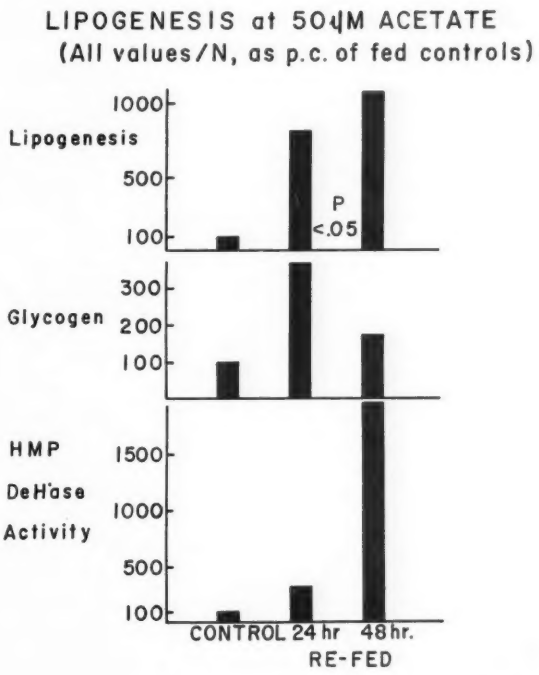

FIG. 6. Lipogenesis, glycogen and shunt enzymes. All values referred to liver nitrogen and expressed as percentage of control. Lipogenesis experiments done with 50 micromols of acetate per beaker.

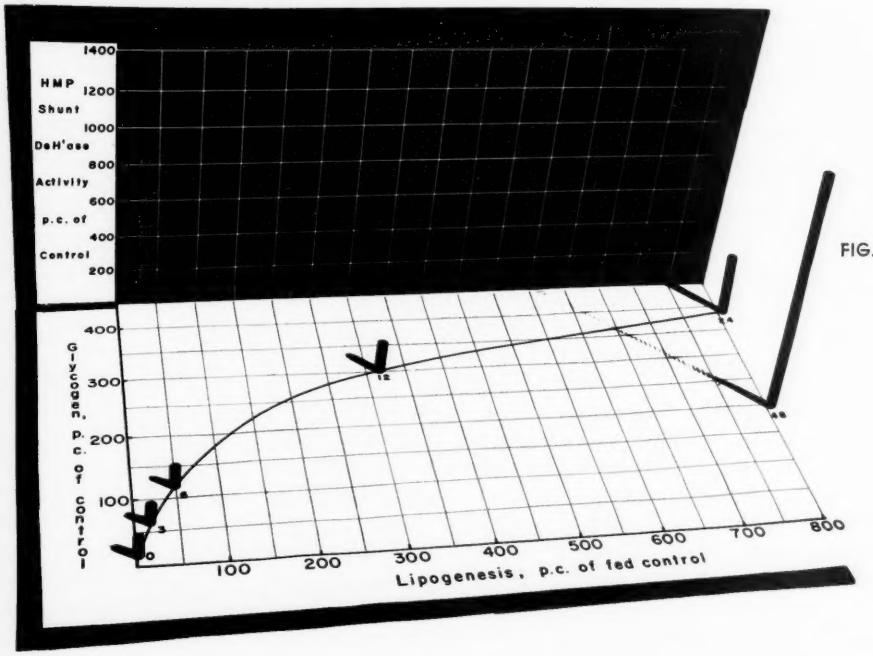

FIG. 5. Hexosemonophosphate shunt dehydrogenase activity, initial glycogen content and lipogenesis by liver slices. All values were referred to liver nitrogen and plotted as percentage of chow-fed control. Numbers at each point represent hours after the beginning of refeeding. See text for additional information.

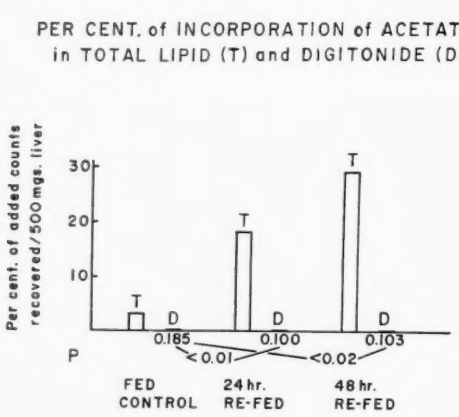

FIGURE 7

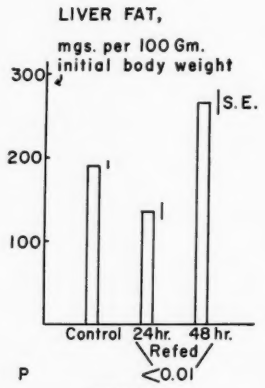

FIGURE 8

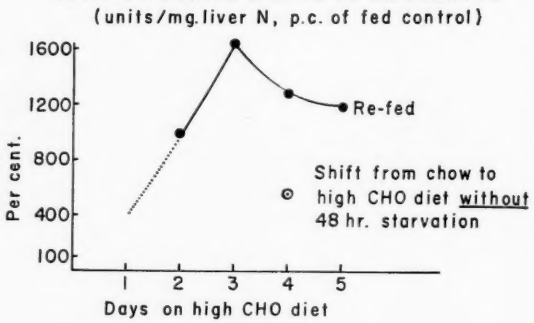

FIGURE 9

ment designed to show the duration of this change. Figure 9 shows that, in a separate series in which no lipogenesis studies were made, the shunt dehydrogenase activity did not reach its peak until seventy-two hours after the beginning of refeeding. Five days after the end of the starvation period the activity of the shunt enzymes was still very substantially above the control level. Since all animals in this study had been on a chow diet until the beginning of the starvation period, one group of six rats was shifted from a chow diet to a high carbohydrate diet without a prior period of starvation. The shunt dehydrogenase activity of the livers of these animals was significantly elevated, but it was well below the levels seen in rats with a history of prior starvation.

DISCUSSION

The large changes in liver weight as a result of starvation and subsequent refeeding are attributable in part to deglycogenation followed by very extensive glycogen deposition. The amounts of glycogen in the livers of twenty-four-hour refed rats were often in excess of 15 per cent of the wet weight of the organ. Table 1 shows that, in the presence of these amounts of glycogen, there was a significant decrease in percentage of liver nitrogen. The mechanisms involved in this extraordinary glycogen deposition are not known. It is possible that the phenomenon may be caused by the combination of realimentation with a high carbohydrate diet (which can cause a significant elevation of liver glycogen levels without prior starvation) and the persistence into the refeeding period of an inappropriately high level of gluconeogenesis initiated during the preceding fast.

Inspection of the time curves for lipogenesis and shunt enzyme activity (figures 3 and 4) shows that the rearrangement of the biochemical machinery of the cell which enables it to synthesize fat very rapidly takes place over a period of hours in much the same way that the recovery of the lipogenic activity of the diabetic liver following insulin[9] or fructose[10] administration requires a period of hours.

The three-dimensional plot of lipogenesis, shunt activity and glycogen (figure 5) reemphasizes the importance of initial liver glycogen as a determinant of the lipogenic performance of liver slices in vitro, particularly during the period of relative constancy of shunt enzyme activity. When the shunt activity is markedly increased, and when the glycogen levels are high, the slices appear to have additional lipogenic activity. The fact that persistently high lipogenic activity was demonstrated when the glycogen fell and the shunt activity increased markedly strongly suggests that lipogenic performance is not determined simply by how much glycogen was in the slice at the beginning of the incubation period, but also by the available enzyme sys-

tems for its metabolism via the hexosemonophosphate shunt. In order for a continuing supply of TPNH to be maintained, a pool of glucose-6-phosphate must be provided, but if substrate traffic over the shunt is maintained at a high level by the presence of a large amount of shunt dehydrogenase, just as large a TPNH supply can be maintained with a smaller initial potential glucose-6-phosphate pool. It should be emphasized that, at the moment, it is a matter of conjecture that the level of shunt dehydrogenase activity as measured in these experiments determines the rate of substrate metabolism via the hexosemonophosphate shunt. Experiments with C_1- and C_6-labeled glucose are now being done to study this problem.

There are many other experiments in which the differentially labeled glucose technic has been used that suggest that (a) in diabetic liver shunt traffic is low[11] and (b) in adipose tissue and in lactating mammary gland high rates of fatty acid synthesis are accompanied by high rates of metabolism via the shunt pathway.[12,13] In the diabetic liver[14] and in the lactating mammary[15] gland good correlations have been shown between hexosemonophosphate shunt enzyme activity and glucose metabolism via the shunt as estimated by the differentially labeled glucose technic. These observations, and those of Siperstein,[16] who was able to stimulate lipogenesis in cell-free preparations by providing TPN reducing systems, generally support the view that such systems play an important physiologic role in adaptive fluctuations in lipogenesis such as the ones described here. It should be pointed out that TPNH is required specifically not only for the reduction of crotonyl CoA but also for ethylene reductions that occur on subsequent two carbon additions all the way up to long chain fatty acids.[6,17]

Siperstein[16] has suggested that TPNH is required for cholesterol synthesis as well as fatty acid synthesis. Therefore it was of great interest to discover whether or not cholesterol synthesis from acetate was enhanced in liver slices in which hexosemonophosphate shunt activity was known to be extremely high. It has already been pointed out that there is a dissociation between cholesterol synthesis and fatty acid synthesis in the diabetic liver,[18] in which the former is high and the latter vanishingly low. In the twenty-four- and forty-eight-hour refed liver a reverse dissociation is apparent, for here (figure 7) the rate of lipogenesis is very high while there is a significant *decrease* in rate of incorporation of the acetate label into the "cholesterol" fraction. Obviously, the factors that influence the rate of fatty acid synthesis in the liver do not necessarily affect the cholesterol synthetic mechanisms in the same direction.

A diagrammatic hypothesis concerning the failure of lipogenesis in diabetes and starvation and the adaptive enhancement of the process in the refeeding phenomenon is presented in figure 10. The variation in width of the arrows in this diagram is not intended to have precise quantitative significance. This is merely an attempt to show how the potential metabolic activity of the individual liver cell is changed by the conditions under discussion and how these changes relate to the chemical economy of the whole organism. While emphasis is placed on the specific ethylene reduction that occurs between crotonyl S CoA and butyryl S CoA, it should be recalled that similar reactions occur on further two carbon fragment addition as the fatty acid is progressively lengthened.[17] In the diabetic cell, gluconeogenesis from amino acids proceeds at a high rate. There is diminished glucose utilization via both Emden-Meyerhof and HMP shunt pathways.[11] This (as Langdon,[9] Siperstein[16] and others have suggested) has the effect of failing to provide a sufficiently large pool of reduced cofactors to support reductive synthesis. That the lack of TPNH is most critical is shown by the failure of butyryl CoA formation[5] while ketone body formation, which is independent of TPNH, proceeds apace. The increase in glucose-6-phosphatase activity that accompanies this condition[19] has the effect of marking glucose produced from amino acids "for export only." This in turn ensures a continuing supply of glucose to tissues like the central nervous system, whose metabolism, not requiring insulin, is mainly dependent on glucose. There is an accentuation of fatty acid catabolism in liver and, probably, muscle by analogy with the adaptation that occurs in fat feeding.[20]

The cell of the refed animal makes a remarkable adaptation to the presence of a plethora of glucose. Refeeding probably diminishes glucose-6-phosphatase activity (by analogy with insulin treatment of the diabetic)[21] but possibly does not immediately "shut off" accelerated gluconeogenesis. At the same time a large amount of glucose is absorbed from the gastrointestinal tract. All of these events collaborate to provide a very large pool of glucose-6-phosphate, much more than the cell can possibly dispose of conveniently at first. Some of the excess goes into an expanded glycogen compartment. With the passage of time, more hexosemonophosphate shunt enzyme activity appears and substrate traffic over the shunt pathway increases. This has the effect of generating increasingly large amounts of TPNH which progressively increases the cell's potential for fatty acid synthesis and thus helps the cell dispose

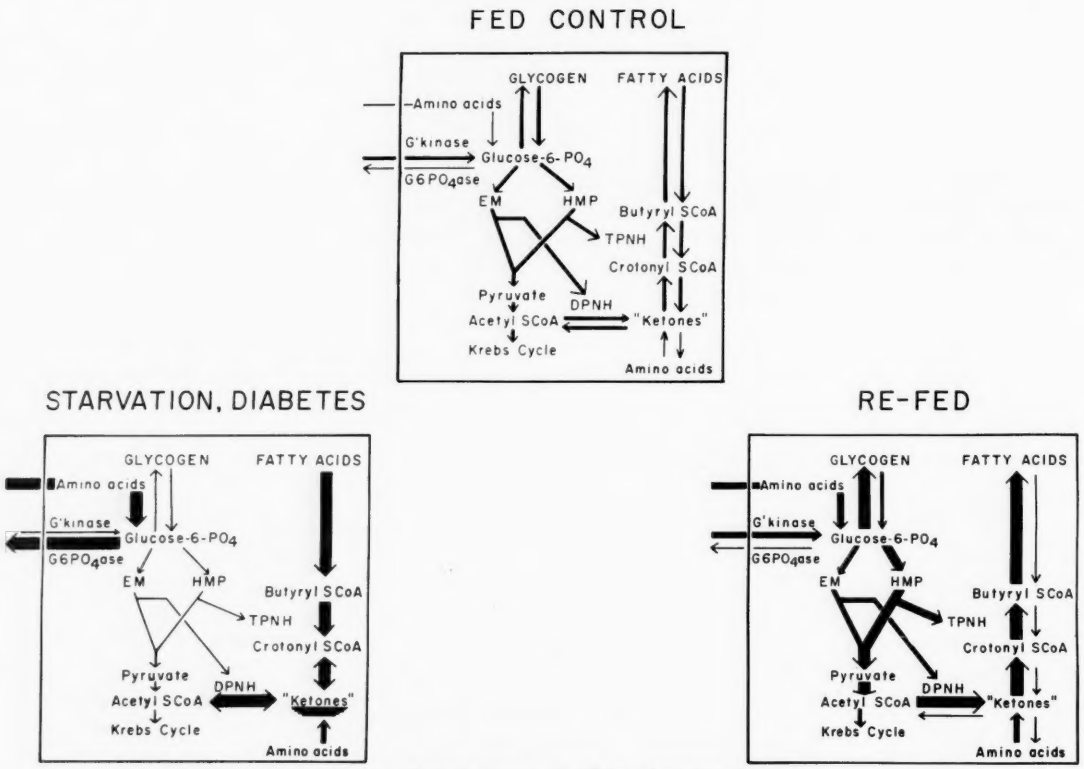

FIG. 10. Hypothetical schemes of cellular adaptation. See text.

of its large glucose load. (There is, as yet, no definite information about the quantities of glucose that traverse the Emden-Meyerhof pathway and are oxidized in the refed liver.) The net effect of the accentuation of the shunt pathway is to facilitate lipogenesis when it is physiologically advantageous for this process to proceed briskly. The repletion of fat stores is especially important for an animal that has depleted them as a result of a forty-eight-hour period of starvation.

The nature of the increase in shunt enzyme activity described here is as yet unknown. We have been careful to refer to this phenomenon as an increase in activity, rather than a de novo synthesis of enzyme, although the latter view represents our current working hypothesis. Certainly, the persistence of high levels of shunt activity over a five-day period shown in figure 9 suggests the possibility that new enzyme has been synthesized. At present, we are inclined to view the shunt pathway as an inducible enzyme system similar to those seen in microorganisms.[22] Possibly, glucose-6-phosphate can function as an inducer. Our current studies are concerned with this hypothesis.

SUMMARY

Liver slices of rats starved for forty-eight hours and refed a high carbohydrate diet for forty-eight hours show extremely high rates of lipogenesis from acetate-1-C^{14} when compared with those of chow-fed controls. In order to test the hypothesis that glycogenolysis may influence the rate of lipogenesis by providing TPNH for critically placed ethylene reductions in the synthesis of fatty acids, rats were starved for forty-eight hours, refed and killed in groups of six at 3, 6, 12, 24, and 48 hours after the beginning of refeeding. Lipogenesis by liver slices, glycogen content, and hexosemonophosphate shunt dehydrogenase activity and other pertinent measurements were made on the same livers. From 0 to six hours lipogenesis correlated well with initial glycogen level, but at forty-eight hours lipogenesis was as high as it had been at twenty-four, though the glycogen had dropped by half. Hexosemonophosphate shunt dehydrogenase activity at this time was more than three times the twenty-four-hour level. The three-way correlation among these variables suggests that substrate traffic over

the shunt may be an important determinant of lipogenesis rate. A hypothetical scheme is presented in which an attempt is made to relate the phenomenon of adaptive hyperlipogenesis to the problem of failure of lipogenesis observed in starvation and diabetes.

SUMMARIO IN INTERLINGUA

Le Shunt De Hexosa-Monophosphato E Hyperlipogenese Adaptatori

Trenchos de hepate ab rattos non alimentate durante quaranta-octo horas e re-alimentate durante quaranta-octo horas con dietas ric in hydrato de carbon revela un extrememente intense lipogenese ab acetato-1-C^{14} in comparation con specimens de controlo. Esseva formulate le hypothese que glycogenolyse exerce un influentia super le intensitate del lipogenese per provider TPNH pro reductiones ethylenic de position critic in le synthese de acidos grasse. Pro testar iste hypothese, rattos non alimentate durante quaranta-octo horas esseva re-alimentate e occidite in gruppos de sex a periodos de 3, 6, 12, 24, e 48 horas post le recomenciamento del alimentation. Le lipogenese per trenchos de hepate, le contento de glycogeno, le activitate de dishydrogenase del shunt de hexosa-monophosphato, e altere pertinente factores esseva determinate in le mesme hepates. Usque a sex horas post le recomenciamento del alimentation, le lipogenese se monstrava ben correlationate con le nivello initial de glycogeno, sed post quaranta-octo horas le lipogenese esseva tanto intense como post vinti-quatro horas, ben que le nivello de glycogeno habeva descendite per un medietate. Le activitate de dishydrogenase del shunt de hexosa-monophosphato a iste tempore esseva plus que tres vices illo trovate post vinti-quatro horas. Le correlation tridirectional inter iste variabiles suggere que migration de substrato via le shunt es possibilemente un factor importante in determinar le intensitate del lipogenese.

Es presentate un schema hypothetic que tenta relationar le phenomeno del hyperlipogenese adaptatori con le problema del collapso lipogenetic que ha essite observate in affamation e in diabete.

ACKNOWLEDGMENT

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The Determination of Blood Sugar

A Rapid Screening Method Utilizing Glucose Oxidase Paper

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Harold L. Dobson, M.D., and James A. Greene, M.D., Houston

Following the isolation of the enzyme glucose oxidase by Müller[1] in 1928 and Coulthard et al.[2] in 1942 from Penicillium notatum and Aspergillus niger, a specific method for the enzymatic determination of glucose became available. Early workers with this enzyme soon evolved methods for the determination of glucose in biologic fluids,[3-6] including blood and urine,[7] which are specific and accurate.

Glucose oxidase is a flavoprotein with a molecular weight of 152,000; alloxazine-adenine dinucleotide is the prosthetic group. The reactions by which glucose is oxidized by glucose oxidase are shown in the following formulae (adapted from Froesch):[7]

a. β- glucose + glucose oxidase → gluconolactone + glucose oxidase H_2

b. Gluconolactone + H_2O → gluconic acid

c. Glucose oxidase H_2 + 2 O → glucose oxidase + H_2O_2

d. H_2O_2 + catalase → $H_2O + O_2$

Over-all Reaction

e. Glucose + ½ O_2 ——→ gluconic acid

The oxidation of β-glucose to gluconolactone (reaction a) involves the reduction of the prosthetic group of glucose oxidase and the hydration of the gluconolactone to gluconic acid (reaction b).

Molecular oxygen acts as a hydrogen acceptor for the re-oxidation of the reduced flavoprotein; the H_2O_2 inhibits the reaction, hence a catalase is necessary to catalyze its decomposition to H_2O and O_2 (reaction d). The over-all reaction results in the incorporation of one atom of oxygen into each glucose molecule, and glucose thereby loses its reducing properties. (See footnote column one.)

In 1956 filter paper impregnated with glucose oxidase, catalyzing enzymes and a color indicator was used clinically for the measurement of glucose in the urine. These products, Tes-Tape and Clinistix, have found extensive use in the detection of glycosuria. Such papers have a wide margin of error and reliance on them in achieving control of diabetes may be unsafe, since very high urine glucose levels may not be detected.[9] Both products have been found capable of detecting amounts of glucose in the urine as low as 0.1 per cent, but their use as a quantitative procedure for urine containing more than 0.5 per cent glucose is considered unsatisfactory. Nevertheless, we have found these products fairly reliable in the detection of asymptomatic glycosuria and particularly useful in screening the urine in a large diabetes clinic population for reducing substances other than glucose. Recently, we have used similar test paper designed to measure blood glucose.*

METHODS

This test material, filter paper in tape form impregnated with glucose oxidase, catalyzing enzyme and a suitable color indicator, is moistened with one drop of finger or ear-lobe blood, gently blotted, and the red color developing at the end of three minutes compared visually with a reference color scale calibrated to correspond to blood sugars of 50, 125, 250, and 350 mg. per cent. One technician performed all of the determinations, and the values obtained have been compared with a simultaneously obtained "true" blood sugar, as determined on finger or venous blood using the Somogyi-Nelson technic (normal values 60-95 mg. per cent).[10] The blood glucose oxidase paper values were determined and recorded before the completion of the Somogyi-

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It has been shown that glucose in solution exists in two forms in the relatively constant proportions of 36 alpha to 64 per cent beta, and that glucose oxidase is virtually specific for oxidation of β-glucose. Studies by Keilin and Hartree[8] have demonstrated in the glucose oxidase preparations the presence of an enzyme, (gluco) mutarotase, which has the ability to convert the glucose rapidly to the β-form.

*Supplied through the courtesy of Dr. W. R. Kirtley, Eli Lilly and Company, Indianapolis, Indiana.

Nelson determination. One hundred and seventy-seven paired observations were obtained. The results utilizing the tape were recorded in absolute values in an attempt to encourage the technician to interpolate readings for color changes falling between the four standard color scale values.

Several members of the house staff at Jefferson Davis Hospital have been given the blood test paper to use in the routine diagnosis and care of patients. These house officers were shown the proper procedures in the use of the test paper, but they received no extensive instructions.

For statistical comparison twenty-nine blood samples were analyzed simultaneously by a modified Folin-Wu[11] and the Somogyi-Nelson[10] procedures.

RESULTS AND DISCUSSION

The manufacturer designed the blood test paper to be used as a rapid method of determining the approximate level of blood glucose. For this purpose a color range from 50 to 350 mg. per cent was selected; therefore the blood paper shows no further change when the blood glucose drops below 50 mg. per cent and there is little increase in color intensity above 350 mg. per cent. It has been our experience that interns, residents and technicians, even those unfamiliar with the test paper, can quickly and easily determine whether a patient's blood glucose is low, normal or elevated. It does take some experience to make a finer differentiation, but with a little experience in reading the more subtle color changes this paper can be used as an accurate measure of the blood glucose level, within the limitations of the color scale.

In figure 1 are shown all 177 observations along with correlation as described by the regression equation. In figure 2 the first fifty-nine (one third) of the observations are shown, while figure 3 shows the last fifty-nine paired observations. It should be emphasized that the technician performing these determinations had no experience with this tape at the start of this series, yet satisfactory accuracy was achieved from the very first. As might be expected the accuracy improved with experience (figure 3).

Statistical analysis, appearing in table 1, further strengthens the impression that the blood tape is a satisfactory method of obtaining a quantitative blood sugar level. As it is known that the blood paper shows satisfactory color changes in the range of 50 to 350 mg. per cent, only those results in which the Somogyi-Nelson value fell within this range were used in these analyses. The results obtained by simultaneous analysis by the

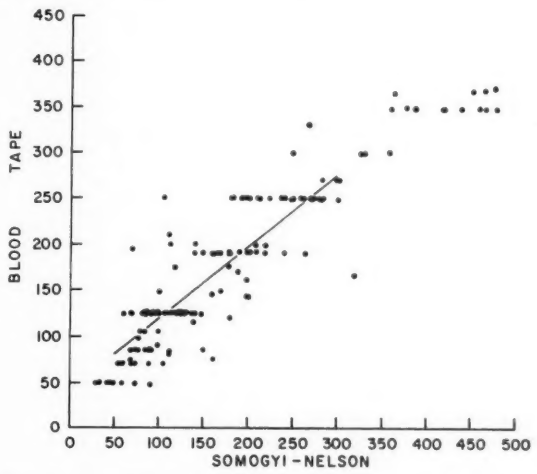

FIG. 1. The relation of the glucose oxidase paper blood sugar level to the simultaneously determined Somogyi-Nelson value in all 177 pairs of observations. The line represents the calculated regression equation.

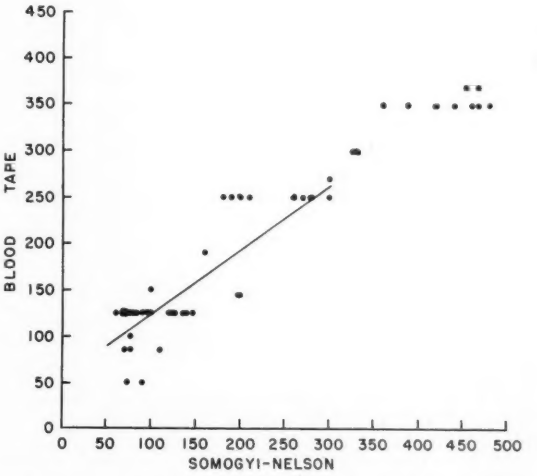

FIG. 2. A comparison of blood sugar levels determined by the glucose-oxidase paper and the Somogyi-Nelson technic (first fifty-nine observations).

Folin-Wu and the Somogyi-Nelson methods were submitted to the same statistical study. In table 1 it may be seen that the correlation coefficient is good in all instances and that the use of the blood glucose paper for quantitative determination is as good as, or possibly slightly better than, the Folin-Wu method (using the Somogyi-Nelson values as the basis of comparison). In addition the standard error of the difference of the mean indicated that the differences in the means be-

TABLE 1

Statistical evaluation of the blood test paper and Folin-Wu methods compared to the Somogyi-Nelson method

	Blood Glucose Oxidase Paper			Folin-Wu
Number of observations	160			29
Mean ± standard deviation	All observations	First One third	Last One third	
Somogyi-Nelson	155±77 mg. per cent	150±86 mg. per cent	163±74 mg. per cent	144±49 mg. per cent
Blood test paper	163±66	165±69	159±59	
Folin-Wu				165±49
Standard error of the difference of the mean	8.05	15.91	12.37	12.87
Correlation coefficient(r) ± S.E.*	0.89±0.01	0.94±0.01	0.94±0.01	0.93±0.01

*Dunn, H. L. *Physiol. Rev.* 9:339, 1929.

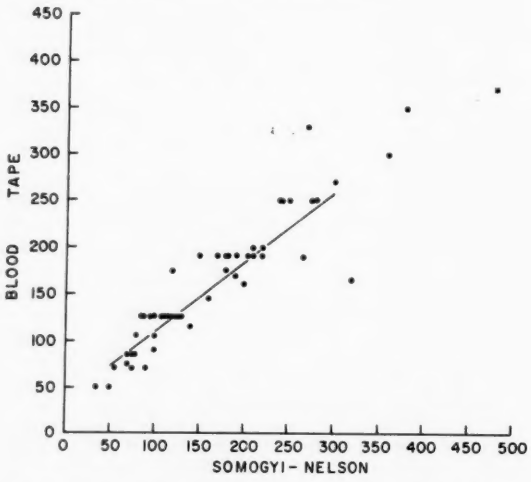

FIG. 3. A comparison of blood sugar levels determined by the glucose-oxidase paper and the Somogyi-Nelson technic (last fifty-nine observations).

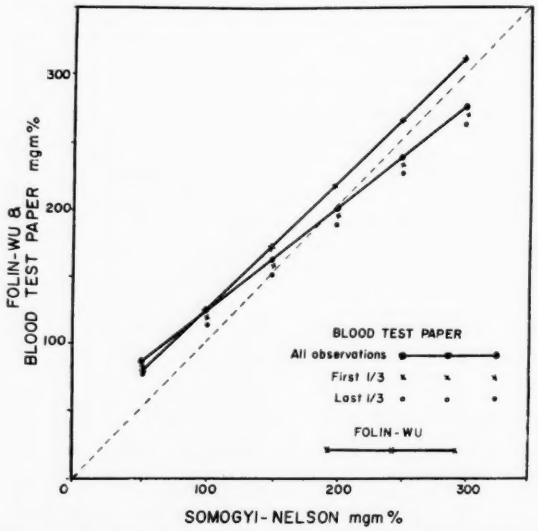

FIG. 4. The lines derived from the regression equations comparing the blood glucose oxidase paper and the Folin-Wu determinations to the simultaneously determined Somogyi-Nelson values. The dotted line represents theoretically perfect correlation.

tween the blood paper and the Somogyi-Nelson methods are probably due to chance alone. A comparison of the curves obtained by means of regression equation is shown in figure 4. This shows the tendency to read the low glucose values at somewhat higher values and the high glucose values at somewhat lower values when the blood test paper is used. In addition, the tendency for the Folin-Wu determination to average 20-25 mg. per cent greater than the Somogyi-Nelson values is also seen.

In addition to the statistical evaluation of the over-all precision there is also the problem of reliability of the tape in the transition of normal to elevated blood sugar levels (100 to 150 mg. per cent). In the first seventy-five pairs of observations there were twenty-three instances in which the difference between the values obtained by the two methods could be of diagnostic significance. Fifteen of these observations were in the 95 to 125 mg. per cent range. Errors here would be of importance mainly in the evaluation of the fasting blood sugar levels. It is obvious that the untrained observer experiences some difficulty in learning to interpolate the color changes in this range. In all instances the paper

was read as showing a level of 125 mg. per cent whereas the Somogyi-Nelson value lay in the 95-100 mg. per cent range. Six determinations had variations of diagnostic significance in the 140 to 200 mg. per cent range, where the variation is of significance in the evaluation of the postprandial blood sugar levels. The remaining two determinations showed such wide variation between the two methods of analysis that it was assumed that errors in analysis were involved.

In the last seventy-five observations there were only four instances in which the difference in the two methods would be of clinical significance, again reflecting the increasing accuracy as experience is gained in the use of the tape. Three of these determinations fell in the 80 to 125 mg. per cent range and one in the 115 to 150 mg. per cent range.

The remarkable degree of correlation is due in part to the consistency of technic employed by a single technician; however, it has been observed that similar accuracy is approached by the ward and staff physicians with very little practice, which bespeaks the reproducibility of the blood test paper method.

This tape has been found to be very useful in the day-to-day management of the hospitalized diabetic, conveniently rapid, and of inconsequent cost compared to the usual methods for the determination of blood sugar. In certain situations, notably diabetic acidosis, postoperative diabetic care, and the obstetric care of the diabetic mother at term, the method has become indispensable. In addition, it has been invaluable in a large charity hospital emergency room as a rapid screening method for differentiating hypoglycemia from hyperglycemia. This is particularly true in comatose patients where head injuries, ethanol intoxication and poisonings must also be quickly recognized and time is a major factor. Recently it has been found that the blood test paper is useful in the rapid diagnosis of episodes of hypoglycemia not originating from insulin therapy, when laboratory facilities have not been available. The regulation of insulin dosage has also been facilitated by this test paper. The only objection is the color scale, which should be expanded to include more color standards if the tape is to be used for quantitative blood sugar determination. The present scale, or one containing even fewer color standards, is adequate where the paper is used only as a screening procedure.

SUMMARY

A new rapid method for the determination of the blood glucose is described that utilizes paper impregnated with the enzyme glucose oxidase. Any person trained in technical observation can utilize this test paper to detect gross alterations in the blood glucose level. With practice and observation the blood glucose oxidase paper can be used as an accurate quantitative method within certain blood sugar ranges. This method is particularly useful in emergencies, in the diagnosis of coma due to extremes in the blood sugar level and as an aid in regulating insulin dosage.

SUMMARIO IN INTERLINGUA

Un Rapide Methodo Pro Le Determination De Sucro Sanguinee, Utilisante Papiro A Oxydase De Glucosa

Es describite un nove e rapide methodo pro le determination de glucosa del sanguine, utilisante papiro impregnate del enzyma oxydase de glucosa. Omne individuo experite in observation technic pote utilisar iste papiro testatori pro deteger grossier alterationes in le nivello del glucosa del sanguine. Post practica e experientia, on pote usar le papiro a oxydase de glucosa sanguinee pro le accurate determination quantitative de sucro sanguinee intra un certe spectro de valores. Le methodo es specialmente utile in casos de urgentia, in le diagnose de coma causate per extremos in le nivello de sucro sanguinee, e in le regulation del dosage de insulina.

ACKNOWLEDGMENT

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Glycosuria as a Cause of Falsely High Hydroxycorticoid Values

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Recently a patient with diabetes mellitus was referred to us with an additional and tentative diagnosis of Cushing's disease. We found an elevated level of urinary free 17-hydroxycorticoids, using the method of Silber and Porter.[1] Further investigation showed that the elevated urinary corticoid values obtained were due to the presence of glucose in the urine, rather than to Cushing's syndrome. The glucose in the urine had interfered with the color produced in the Silber-Porter method for determination of hydroxycorticoids, causing falsely high values. Observations concerning the nature of this interference, together with a method for correcting it, are reported because this method is widely used for measuring urinary hydroxycorticoid levels and because of the fact that glycosuria of a degree which will produce falsely high values is commonly found. Furthermore, glycosuria is particularly common in the group of patients suspected of having Cushing's disease, since this may be a sign suggesting that disease.

Silber and Busch have shown that the presence of 0.1 per cent glucose did not affect this determination.[2] More recently, Louis et al.[3] have shown that specimens containing a greater amount of glucose give falsely high values when β-glucuronidase is used in the determination of hydroxycorticoids by the Silber-Porter method. They postulated that this was due to a chloroform soluble degradation product of glucose produced by the β-glucuronidase. In their preliminary communication, they did not mention whether they had been able to isolate and identify this breakdown product or whether, on the basis of their data, they merely postulated that such a compound might exist.

For the past two years, we have determined urinary free hydroxycorticoids the way in which it was performed by Louis et al., except that ten times as much urine was used for the extraction procedure, and β-glucuronidase is not used. We have found that glycosuria will produce falsely high values for the determination of free and conjugated hydroxycorticoids.

From the Veterans Administration Hospital and the Department of Medicine, University of Oklahoma, Oklahoma City, Oklahoma.

METHOD

Twenty-four specimens of urine were obtained from hospitalized patients with diabetes mellitus, and from nondiabetic patients without known adrenal disease. These specimens were collected in clean bottles without an added preservative and without refrigeration. After collection, the volume was recorded and the intensity of glycosuria was estimated using paper impregnated with glucose oxidase.* An aliquot was saved and kept refrigerated until the determination. The free and total hydroxycorticoids, with and without added sodium bisulfite, were determined in each specimen. The total hydroxycorticoids were determined by the method of Silber and Porter,[1] using β-glucuronidase.† The urine was adjusted to *p*H 6.5 and the six aliquots of 1 ml. were incubated for nineteen hours with 100 Sigma units of β-glucuronidase. One ml. of a solution of sodium bisulfite, 250 mg./ml., was added before incubation to three aliquots, and was added after incubation but before chloroform extraction to three aliquots of each urine specimen. The free hydroxycorticoids were determined by the method of Silber and Porter[1] using 10 ml. aliquots of each urine specimen. One ml. of the solution of sodium bisulfite, 250 mg./ml., was added to three aliquots of each urine specimen from a diabetic patient. The free hydroxycorticoids were also determined without adding sodium bisulfite. All urine specimens were extracted with redistilled CP grade chloroform.

In order to determine if glucose will dissolve to any extent in a watery solution of chloroform, a 2 per cent solution of glucose was extracted in the same way as the aliquots of urine. These chloroform extracts were chromatographed using a butanol, pyridine, water system, (6: 4: 3).

RESULTS

Table 1 shows the results obtained when glucose was added to urine obtained from a patient who did not have diabetes. The twenty-four-hour urine specimen contained

*Tes-Tape—Eli Lilly and Company.
†Nutritional Biochemical Company.

TABLE 1

Effect of added glucose on the results of the Silber-Porter determinations of free hydroxycorticoids

Per cent glucose	0.0	0.1	0.25	0.5	1.0	2.0	3.0
Hydrocortisone standard 12 μg.	12 μg.	12 μg.	12.3 μg.	13.7 μg.	16.3 μg.	19.4 μg.	22 μg.
Normal urine	0.52 mg.	.052 mg.	0.58 mg.	1.15 mg.		2.42 mg.	
Normal urine plus sodium bisulfite	0.52 mg.			0.52 mg.		0.52 mg.	

0.52 mg. of free hydroxycorticoid and an apparent amount of 1.15 mg. when 0.5 per cent glucose was added. When 2 per cent glucose was added, the apparent amount of free hydroxycorticoid was 2.42 mg. This shows that increasing amounts of glucose cause a progressive apparent increase in the amounts of free hydroxycorticoids.

Table 2 shows typical values obtained from diabetic patients with varying degrees of glycosuria. All hydroxycorticoid values were either in the higher range of normal or were abnormal. The normal range in this laboratory is 0.2 to 0.7 mg./24 hr. (Mean ± 2 SD). The addition of sodium bisulfite resulted in values which were in each instance markedly lower than the previous values obtained on the same specimens. These corrected values were all within the normal range, except in Subject 6 who was receiving cortisone therapy. It is also apparent from the data in this table that the degree to which the value was falsely high was only approximately related to the intensity of glycosuria, as estimated by using glucose-oxidase impregnated paper.

TABLE 2

The effect of adding sodium bisulfite to specimens of urine containing glucose before determining the free hydroxycorticoids by Silber-Porter method

Diabetic Subjects	Glucose*	24-hour Hydroxycorticoid with† $NaHSO_3$	without $NaHSO_3$
1	trace	.336 mg.	.673 mg.
2	+3	.298 mg.	.705 mg.
3	+3	.492 mg.	.882 mg.
4	+3	.310 mg.	.480 mg.
5	+4	.686 mg.	1.14 mg.
6	+2	.902 mg.	1.67 mg.
7	trace	.28 mg.	.67 mg.
8	trace	.30 mg.	.76 mg.

*Estimated using Tes-Tape—Eli Lilly.
†250 mg. added to 10 ml. aliquot.

A similar effect was produced when glucose was added to the urine before the determination of total hydroxycorticoids using β-glucuronidase. In a patient with diabetes showing a 2 per cent sugar, the apparent hydroxycorticoid level decreased from 12 mg. to 3.3 mg. when the procedure was performed after the addition of sodium bisulfite.

Table 3 shows that adding sodium bisulfite to specimens of urine before incubation with β-glucuronidase produces falsely low hydroxycorticoid values, while adding sodium bisulfite after incubation produces no effect. It may be noted that the addition of glucose to achieve a level of 2 per cent produced apparent elevations in the hydroxycorticoid values which were corrected by the addition of sodium bisulfite after incubation with β-glucuronidase.

TABLE 3

Effect of added glucose and sodium bisulfite on the apparent value of total urinary hydroxycorticoids by Silber-Porter method

	24-hour 17-Hydroxycorticoid in Mg.	
Control	2.4	3.6
$NaHSO_3$ added before incubation with B-glucuronidase	0.8	—
$NaHSO_3$ after incubation with B-glucuronidase	2.3	—
Glucose Added to Urine to Make a 2 Per Cent Solution		
Control	4.4	5.8
$NaHSO_3$ pre-incubation	1.6	—
$NaHSO_3$ postincubation	2.6	4.2

Figure 1 shows the absorption spectrum of the Silber-Porter chromagen following the addition of glucose. Also shown is the spectrum produced when glucose is allowed to react directly with phenylhydrazine reagent used in this determination. This absorption spectrum shows a peak below 375, whereas the color produced when this procedure is performed in the absence of glucose shows a peak at 410 mu. This confirms the absorption spectrum obtained by others.[4,5] However, when glucose was extracted from a watery solution with chloroform, analysis revealed a peak at 460 mu. After extraction of glucose and cortisone, a peak at 410 was obtained. However, the entire curve had shifted upward and a secondary poorly defined peak appeared at 460 mu. The substantial differences in the spectra produced when glucose was extracted with and without chloroform indicate clearly that a new compound had been

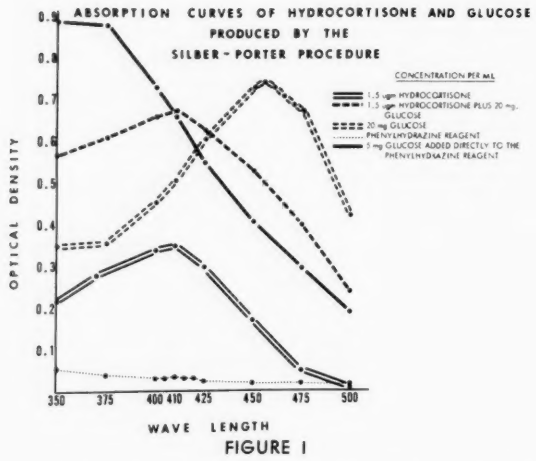

FIGURE 1

formed during the extraction procedure, and that the falsely high hydroxycorticoid values obtained in the presence of glucose were not due to glucose itself.

A 2 per cent solution of glucose was extracted by chloroform, as in the Silber-Porter reaction, and the chloroform extract was then chromatographed to determine the presence or absence of glucose. No glucose was present on the chromatogram.

DISCUSSION

The Silber-Porter reaction is widely used as a measure of hydroxycorticoids. Many of the specimens, which are analyzed with this procedure, contain glucose, since glycosuria is common in patients suspected of having Cushing's syndrome. Louis et al.[3] first called attention to the fact that glucose would interfere with the determination of conjugated hydroxycorticoids. They postulated that the β-glucuronidase used by them produced during incubation a chloroform soluble breakdown product of glucose.

The results presented here show this could not be the cause of the elevated values, since glucose interferes with this determination even in the absence of β-glucuronidase. That glucose itself does not produce the interference is indicated by the fact that glucose was not present on the chromatogram. Furthermore, the absorption spectrum of the glucose phenylhydrazine was different from that of the compound which caused the interference.

We have not determined the chemical formula of the compound, which interferes with this determination. However, it would appear that it is a derivative of glucose. In the presence of light, small amounts of hypochlorites and other substances are formed from chloroform. Commercial grade chloroform contains ethyl alcohol to prevent this reaction. It is possible that these breakdown products react with the glucose to produce a chloroform soluble product which will produce color.

As suggested by Louis et al.[6] the addition of sodium bisulfite, 250 mg./ml., will prevent this reaction. The data obtained here would suggest that if the sodium bisulfite is added after incubation with β-glucuronidase correct values of total hydroxycorticoids are obtained. If on the other hand, it is added prior to incubation, falsely low values are obtained.

SUMMARY

1. The presence of glucose in the urine, in amounts exceeding 0.25 per cent, will result in falsely high values of free and of conjugated hydroxycorticoids, when the Silber-Porter method is used.

2. This interference can be prevented by adding sodium bisulfite to the urine, prior to the extraction with chloroform.

SUMMARIO IN INTERLINGUA

Glycosuria Como Un Causa De Falsemente Alte Valores Hydroxycorticoide

1. Le presentia de glucosa in le urina in quantitates de plus que 0,25 pro cento resulta in falsemente alte valores pro libere e conjugate hydroxycorticoides quando le methodo Silber-Porter es usate.

2. Iste disturbation pote esser prevenite per le addition de bisulfito de natrium al urina, ante le extraction con chloroformo.

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Diabetic Sciatic Neuropathy

Report of a Case

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It is a well-established fact that patients with diabetes mellitus frequently show manifestations of neurological changes.[1-3] These are most commonly related to the peripheral nerves and are usually bilateral and symmetrical. In a large series of cases reported by Goodman et al., there were only two cases of sciatic neuropathy, or 0.8 per cent of their series.[4] In the two cases described by Goodman et al., the involvement appeared to be in the peripheral portions of both sciatic nerves or their branches. The following case is reported because it was unilateral and involved the whole sciatic nerve.

CASE REPORT

Mrs. A.D., age fifty-nine, was first examined on July 25, 1956. At that time, she gave a history of having had diabetes for twenty years, during which time glycosuria had been controlled by diet alone. During the prior seven to eight months, she had been taking 30 units of Protamine Zinc insulin daily. Following the institution of insulin therapy, she noted pain in the left hip which radiated to her toes, and numbness of the left leg and foot. This became progressively worse and developed into a severe burning sensation over the left leg, most painful in the early morning hours. A month after the onset of these symptoms, she was treated in the hospital for a brief time with diathermy and bed rest with no relief. There was no change in the pain with bending, coughing or sneezing, and there was no history of back trauma. The patient admitted that she did not adhere to her diet although she did take insulin regularly and was apparently aglycosuric.

The patient's general physical examination was within normal limits. The neurological examination revealed her to be right-handed, alert and co-operative. She walked with a slight limp of the left lower extremity. The examination of the skull and spine, cranial nerves, co-ordination, stereognosis and speech was not remarkable. There was diffuse atrophy of the left calf, thigh and buttocks. There was weakness of all the muscles of the left lower extremity, especially the peroneals, extensor hallucis longus and the tibialis anterior muscles. The left knee jerk and ankle jerk were barely elicited. Straight leg-raising tests were to 90 degrees from the table on the right and 70 degrees on the left. The skin over the lower extremity was very dry and atrophic. There was diffuse superficial hyperesthesia of the left lower extremity, except for the area of the peroneal nerve over which there was hypesthesia.

The patient was hospitalized again for three weeks during which time it was found that the blood counts, urinalysis and sedimentation rate were normal. Her fasting blood sugar was 132 mg. per 100 ml. X-ray films of the skull and lumbar spine were within normal limits, but those of the left foot showed some osteoporosis. A lumbar myelogram was done and showed a normal opening pressure and manometrics with a total protein of 44 mg. per 100 ml. The X rays revealed a filling defect of the left root sleeve at L-4 and a questionable one at L-5. She was treated with bed rest, physiotherapy, vitamins, Meticorten and ganglionic blocking agents. There was no change during her stay and she complained constantly of the burning pain in her legs.

The patient was discharged unimproved; she became unable to walk due to her severe pain. She was then referred to a diabetes clinic where she was examined and her X rays reviewed. It was felt that her difficulty was not due to the questionable herniated disc. Another lumbar puncture was within normal limits. At that time, she showed general atrophy of the left foot, calf and thigh which had a striking weakness, and also a slight weakness of the left quadriceps muscle.

She was discharged with directions to take vitamin B_{12} 1,000 mcg., three times a week by injection and to maintain a diet of 140 gm. of carbohydrate, 77 gm. of protein and 76 gm. of fat.

The patient then began to follow her diet more conscientiously and to exercise her lower extremities and back as she had been directed. Within six months, she had made a remarkable improvement. The pain was greatly diminished and the motor function was improving. At the end of one year, there was continuous, although slow, improvement in her walking without a marked change in her neurological picture. Since being seen at the clinic, she has been taking 24 units of NPH insulin mixed with 7 units of crystalline insulin.

COMMENT

This patient illustrates several interesting points. Although she had known diabetes for twenty years, her complaints of paresthesias and pain started only shortly after she began taking insulin regularly. Other important features were the unusually severe neuropathy, predominantly of the sciatic nerve, and the fact that it was only unilateral. In the differential diagnosis the possibility of a spinal cord root compression syndrome arose, but the filling defect of the myelogram was not adequate to explain the patient's extensive involvement. This case also demonstrates that the prognosis in such patients is good, if they adhere to a proper diet and at the same time rehabilitate the involved muscles.

SUMMARY

A case of a unilateral extensive sciatic neuropathy oc-

curring in a fifty-nine-year-old woman with diabetes of twenty years' duration is presented. Her symptoms appeared shortly after institution of insulin therapy. In spite of the extensive motor damage and severe disability due to hyperesthesias, she made a gradual but definite recovery on a regime of a carefully controlled diet, vitamin supplements and therapeutic exercises.

SUMMARIO IN INTERLINGUA

Neuropathia Sciatic Diabetic: Reporto De Un Caso

Es presentate un caso de extense neuropathia sciatic unilateral occurrente in un femina de cinquanta-nove annos de etate qui habeva diabete de un duration de vinti annos. Su symptomas se manifestava brevemente post le institution del therapia a insulina. In despecto de extense damnos motori e de sever grados de invaliditate in consequentia de hyperesthesias, illa se restabliva gradualsed definitemente con un regime de cautemente regulate dieta, vitaminas supplementari, e exercitios therapeutic.

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EDITORIALS

PREGNANCY AND DIABETES

Many factors contribute to fetal wastage in diabetes and multiple-part programs ensure the highest possible salvage.

The causes of fetal loss may be classified as three: preventable, unpreventable and those demanding maximum research exploration. The preventable fetal losses include the early spontaneous abortions; the intra-uterine deaths due to ketoacidosis, to toxemia, to polyhydramnios, and to some lesser degrees of vascular insufficiency; the premature losses due to polyhydramnios; and the intrapartum deaths due to the disproportion between the abnormally wide shoulders of the infant and the pelvis.

The unpreventable causes include the extreme degrees of vascular insufficiency, congenital anomalies and the accidents such as knotted cords, placenta previa and ruptured uteri, which would be equally devastating in the general population.

Deserving and currently receiving maximum research exploration are the neonatal losses due to pulmonary hyaline membranes.

No specific agent alone, but many forms of combined therapy increase the chances of fetal survival.

Chemical control of diabetes is the only means of preventing intra-uterine deaths due to ketoacidosis, during which fetal loss approaches 100 per cent. Fetal susceptibility to ketoacidosis is greatest in the second trimester. Patient susceptibility is highest in adult-type diabetes of short duration. Intensification of diabetes in this group, inexperienced in the management of malignant diabetes, may be disastrous. Ketoacidosis may be physiologically induced by the placental production of diabetogenic hormones, but sometimes it is iatrogenically induced by the prescription of diets inadequate in both calories and carbohydrate. Its progress may be checked too slowly by suboptimal insulin therapy. The requirement for insulin in ketoacidosis in pregnancy is disproportionately great compared with the blood glucose level or CO_2 content. Weekly prenatal visits, however, with immediate revision of insulin and dietary regimens guarantee prevention of ketoacidosis.

Sodium restriction and diuretic therapy control the degree of but do not eliminate the occurrence of hydramnios. However, premature deliveries due to early rupture of membranes by polyhydramnios are prevented.

Female sex hormonal therapy is the main defense against spontaneous abortion and some premature deliveries. Its greatest service in diabetic pregnancies is in the prevention of toxemia with its high incidence of intra-uterine deaths. The rates of these accidents in series where such endocrine therapy is used in adequate doses are negligible.

Early timing of the delivery remains an important part of management. It is directed against intra-uterine deaths due to vascular insufficiency in any pregnancy. In the diabetic, three areas are involved. Vascular changes producing obstructive vascular disease are demonstrable in the pelvis, uterus and the placenta. Ab-

normal vasomotor responses, venular dilatation and arteriolar constriction seen in the bulbar conjunctival vessels of diabetics are exaggerated in the pregnant diabetic. Similar changes in uterine, placental and pelvic vessels are inferred. Although poorly judged timing and errors in dates may turn intra-uterine into neonatal deaths, early timed deliveries have lowered viable mortality rates.

Pelvic deliveries are admittedly desirable, but failure of progress after induction, late diagnosis of disproportion, and breech presentation indicate the need for cesarean section. Such programs eliminate intrapartum deaths completely.

In part because of prematurity, in part because of cesarean sections, but mostly because of diabetes, pulmonary hyaline membranes are the most important cause of morbidity and mortality in the neonatal period. The early time of appearance of symptoms and signs suggests a prenatal influence; the demonstration that the protein is fibrin suggests an intravascular origin.

The 3 per cent mortality due to congenital anomalies can perhaps be lowered by avoiding such causes of anoxia as acidosis and hypoglycemia. Prevention of those due to chronic vascular insufficiency will depend upon the preservation of better vascular states prior to pregnancy onset.

Much confusion exists today because of exaggerated claims. These are largely two: one that good treatment of diabetes alone guarantees fetal survival; or, two, that female sex hormones guarantee salvage in spite of poor control of diabetes, late timing of delivery and omission of a diuretic program.

Constant supervision and dietary control in revealed prediabetes has given a fetal salvage higher than that of the general population. The combination of chemical control of diabetes with diet and insulin, the use of diuretics, the use of female sex hormones, early timing of the delivery and immediate care of the newborn infant has resulted in nondiabetic salvage in Class B patients (adult at onset, short-term without vascular lesions). As maternal vascular lesions progress, fetal salvage decreases until in cases with the severest grade, nephropathy (Class F), fetal salvage occurs in only half the patients.

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THE "KIMMELSTIEL-WILSON LESION"

Over twenty years ago Kimmelstiel and Wilson[1] described, in the kidneys of diabetics, nodular glomerular lesions which have come to be known as "intercapillary glomerulosclerosis" or "Kimmelstiel-Wilson lesions." Further clarification and indications that these changes might be specific for diabetes came from the studies of Allen,[2] Robbins et al.[3] and many others. A "diffuse" glomerular lesion, sometimes associated with, and possibly preceding the nodular, has been described by Bell[4] and has been given increasing recognition in recent years. However, although recent studies[5] suggest that the diffuse lesion may be more closely related to clinical symptoms than the nodular, it must be emphasized that the term "Kimmelstiel-Wilson lesion" is properly applied only to the classical nodular lesion described by the original authors.

In the past six years there has been a revival of interest in another type of lesion, not specific for diabetes and probably of minor importance, which was described, in at least one form, by the original authors[1] but not emphasized by them. This has been called "the exudative lesion" by British workers[6,7] and has also been described in some detail by Koss[8] and Barrie et al.[9] It consists of a mass of homogeneous "fibrinoid" material, differing in texture, tinctorial qualities and location from the classical hyaline nodular lesion of Kimmelstiel and Wilson.[10] It is characteristically located either in the lumen of a capillary or in the capsular space, in contrast to the Kimmelstiel-Wilson lesion, which is intercapillary or intramural, depending on one's concepts of the "mesangium," intercapillary space and basement membrane of the glomerulus. The exudative lesion can be distinguished in the electron microscope by its density as well as its location.[11] It is usually a late or terminal phenomenon in diabetic nephropathy and is clearly nonspecific for diabetes, since typical examples can be found in other diseases, such as chronic glomerulonephritis.[12]

The nodular and exudative lesions resemble each other only superficially in that each appears as a mass of eosinophilic material; otherwise, as indicated above, they are quite different. Unfortunately, perhaps, for readers who are not accustomed to looking at histological sections of diabetic kidneys, a clear distinction between the two is not always made in current literature. For instance, it is stated that "Kimmelstiel-Wilson type lesions" were found in a series of nondiabetics and that this constitutes evidence that "the Kimmelstiel-Wilson lesion is not absolutely specific for diabetes mellitus"[13] whereas in fact the illustrations and descriptions presented refer to the exudative lesion and not the nodular lesion of the original authors.

Further difficulty occasionally arises in the description

of lesions induced in the kidneys of rabbits by large doses of cortisone or related steroids, when, for example, it is said that the lesions "resemble those of human diabetic glomerulosclerosis,"[14] whereas the lesions induced by these steroids are clearly of the nonspecific exudative type[15] and have not been shown to have any definite relation to diabetes. Indeed, superficial resemblances between the cortisone-induced and the human diabetic lesion seem to have been largely responsible for speculations, now increasingly doubted,[16] concerning a possible role of the adrenal cortex in the pathogenesis of renal disease in diabetes. It is, of course, conceivable that the exudative lesion in man may in fact be associated with adrenal cortical activity and yet have no direct relation to diabetes.

There are thus three glomerular lesions which may be found in the diabetic kidney (to say nothing of the commonly present arteriolar sclerosis and pyelonephritis) which need to be clearly differentiated both histologically and semantically if confusion is not to arise.

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Eugene L. Opie

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In 1901 Eugene L. Opie, then twenty-eight years of age, first reported the pathological lesions of the islands of Langerhans in diabetic man. His published papers were titled "The Relations of Chronic Interstitial Pancreatitis to the Islands of Langerhans and to Diabetes Mellitus"[1] and "The Relation of Diabetes Mellitus to Lesions of the Pancreas: Hyaline Degeneration of the Islands of Langerhans."[2] These have become accepted as classics in the scientific literature of diabetes and are associated in the minds of investigators in this field as the sequels to the paper of von Mehring and Minkowski[3] in 1890 on the relationship between the pancreas and diabetes and as the precursors of Banting and Best's famed paper announcing the discovery of insulin in 1922.[4]

Eugene Opie's interest in the islands of Langerhans was stimulated by his curiosity during his student days at Johns Hopkins. In Opie's own account written in an article titled "The Peripatetic Education of a Pathologist"[5] he states, "During the laboratory course in pathology I found in a class section of the pancreas a strange body which I showed to Dr. Welch when he made his unhurried round of the class. He told me it was an island of Langerhans described in an inaugural dissertation.

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" 'Find out all you can about these islands of Langerhans,' he said.

"Langerhans' dissertation was accessible in the Surgeon General's Library. Several years later I published studies of the relation of these islands of the pancreas to diabetes mellitus which pointed the way to the endocrine control of the disease."

Opie's first publication on the islands of Langerhans was in 1900. This was titled "On the Histology of the Islands of Langerhans of the Pancreas."[6] Another paper titled "The Pathological Changes Affecting the Islands of Langerhans of the Pancreas"[7] was published in the same year. In his article in the *Archives of Pathology* on "An Inquiry into Certain Aspects of Eugene L. Opie,"[8] Peyton Rous said, "It is plain from Opie's early papers that he started to discover by asking himself urgently what each and every thing meant that came under his eye. When examining postmortem material he was no recorder of final states; the changes met in the dead had for him the livest of implications. He was an inquirer into riddles, who sought after the meaning of diseased organs much as one might seek for the answers to charades. It was with this approach to the unknown that he observed in postmortem sections from the pancreas of a diabetic person the nearly complete destruction by hyaline change of the islands of Langerhans. The hint was enough. He had proved to himself while yet a medical student that the islands of Langerhans were not modified or undeveloped acinous cells, as was currently thought, and had discriminated between the interlobular and interacinous types of chronic interstitial pancreatitis. Now he went on to demonstrate by the perceptive collection of instances that severe injury to Langerhans' islands is followed by diabetes." These papers of Opie's relating diabetes mellitus to chronic interstitial pancreatitis and to hyaline degeneration of the islands of Langerhans[1,2] were published in 1901.

In another autopsy case Opie found hemorrhagic pancreatitis and fat necrosis associated with a stone blocking the ampulla of Vater. This suggested to Opie that the causative agent of the pancreatic damage was the bile diverted into the pancreatic duct by the impacted stone. Experimental proof of this theory was soon forthcoming and Opie published this evidence in 1901 in a paper titled "The Relation of Cholelithiasis to Disease of the Pancreas and to Fat Necrosis."[9]

In the years between 1901 and 1904 Opie carried on extensive research into diseases of the pancreas. His work during these years gained him a recognition as a world-wide authority. His publications during these three years were titled "Etiology of Acute Hemorrhagic Pancreatitis,"[10] "The Causes and Varieties of Chronic Interstitial Pancreatitis,"[11] "Disease of the Pancreas: Its Cause and Nature,"[12] "Disease of the Pancreas: The Symptoms and Treatment of Disease of the Pancreas,"[13] "The Anatomy and Histology of the Pancreas,"[14] "The Anatomy of the Pancreas"[15] and "Lesions Peculiar to the Pancreas and Their Clinical Aspect."[16]

Eugene Lindsay Opie was born in Staunton, Virginia, in 1873. Five years after graduation from medical school he married Gertrude Simpson, the daughter of Dr. Thomas Simpson of Montreal. Four children were born of this marriage. Gertrude Opie died in 1909 and in 1916 Opie married her sister, Margaret. Dr. and Mrs. Opie live in Manhattan.

Dr. Opie's forebears came from Cornwall, England. They left there in 1660 to settle in Virginia at the junction of the Potomac and Yeocomico rivers. Eugene Opie's father was Dr. Thomas Opie of Staunton, Virginia. His mother was Sally Harmon also of Staunton. Dr. Thomas Opie was a graduate of the Medical School of the University of Pennsylvania in the class of 1861. It may be of some interest to note and perhaps indicative of Thomas Opie's open-mindedness and curiosity that after his graduation, on his way south to enlist in Stonewall Jackson's army as a surgeon, he stopped over in Washington to attend Lincoln's inaugural ball. After the war Thomas Opie moved to Baltimore and entered practice. He became one of the founders and later Dean and Professor of Obstetrics and Gynecology of the College of Physicians and Surgeons in Baltimore.

Eugene L. Opie received the degree, Bachelor of Arts, at Johns Hopkins in 1893 and started medical school at the College of Physicians and Surgeons in Baltimore where his father was Dean. He transferred in his second year to Johns Hopkins Medical School and was graduated in 1897 with the first class of that school. Next to Opie's father, no one had more influence on the development of his scientific career than did one of his teachers at Johns Hopkins, William H. Welch. After graduation Dr. Opie became an Associate in Pathology in Dr. Welch's laboratory and remained there until 1904 when he left, at the invitation of Simon Flexner, the Director of the Rockefeller Institute, to become one of the original staff members along with Samuel J. Meltzer, Hideyo Noguchi, J. E. Sweet and P. A. Levene.

In 1910 Opie became Professor of Pathology at Washington University School of Medicine, St. Louis, and served as Dean of that school from 1912 to 1915 during its period of reorganization. On the occasion of the dedication ceremony of the Washington University Medical School Opie said, "The school cannot afford to

neglect any means by which the benefits of medical knowledge of today may be transmitted through its students and its teachers to popular use. Its first purpose will be to make available to them the knowledge of today and it must make sure that they obtain a preparation which will fit them to acquire new knowledge of the future. The ultimate success of the school will depend upon the relatively small number of unusually able men who will become fitted either as practitioners or as teachers to take the highest places in medicine. The habit of keeping pace with the development of knowledge may be acquired by attempting to add to knowledge. The teacher who has contributed to a science has an immense advantage when he attempts to inspire others with a love of truth. Investigation is founded upon the capacity to express reality and is opposed to pretense, egotism and contention. All who have the success of this institution at heart will continue to foster a spirit of loyalty to the purposes which they support. Remedial and preventive medicine represent only one phase of exact knowledge, but the establishment of an institution devoted to the pursuit and dissemination of truths intimately related to individual and social welfare cannot fail to become a significant contribution to the intellectual life of a community in which it is established." G. Canby Robinson in his recent book "Adventures in Medical Education"[17] said of Opie, "He took a leading part in establishing high standards of teaching and in cultivating true university spirit of scholarship in medicine."

During the First World War Opie served with the United States Army Medical Corps in France. Later he served on commissions to study trench fever, pneumonia and influenza.

Opie left St. Louis in 1923 to accept an appointment as Professor of Experimental Pathology at the University of Pennsylvania and Director of the Laboratories at the Henry Phipps Institute for the study, treatment and prevention of tuberculosis. Later he also was appointed Professor of Pathology and Director of the Department of Pathology of the medical school.

In 1932 he became Professor of Pathology at the reorganized Cornell University School of Medicine and Pathologist to the New York Hospital. In 1939 he was appointed visiting Professor of Pathology in the Peiping Union Medical College during the period of the Japanese occupation. Mrs. Opie accompanied Dr. Opie on this trip to China. In 1941 Opie became Professor Emeritus at Cornell. He then returned as a specially invited guest to the Rockefeller Institute to pursue his research interests.

During the second World War, Opie returned to Cornell to help with the teaching. He also acted as part-time visiting Director of the Henry Phipps Institute of the University of Pennsylvania. At the conclusion of these responsibilities Opie returned to the Rockefeller Institute where he is presently busy pursuing his research interests as an Affiliate of the Institute.

During his period of research activities at the Rockefeller Institute, 1904 to 1910, Opie began to study leukocytic enzymes. He identified leukoprotase and carried out studies on anti-enzymes. This led him to study the enzymes of the epitheloid cell and to look into the inflammatory processes involved in tuberculosis. While at the Rockefeller Institute Opie served as co-editor of the *Journal of Experimental Medicine* and became editor of the *Proceedings of the Society for Experimental Medicine and Biology*. He was a founding member of the Harvey Society.

From 1910 to 1923 while at Washington University, St. Louis, Opie interested himself in studying the influence of diet in warding off liver necrosis produced by toxic substances. He then became interested in the problem of tuberculosis and studied the changes in the incidence and character of tuberculosis produced by preceding infection. He also studied the pathological changes seen in children with tuberculosis as compared to the changes seen in adult patients.

From 1923 to 1932, while at the Henry Phipps Institute of the University of Pennsylvania, Opie's researches and activities are well described by Esmond R. Long[18] who succeeded Opie as Director of the Phipps Institute. "I believe there will be universal agreement at the Phipps Institute that Opie combined the scientific disciplines of epidemiology, pathology, clinical medicine and public health in a manner that had never been accomplished before. Under his wise guidance a development of dispensary practice was brought about which at the same time improved the effectiveness of medical service to the public and added a rich source of material for study. The studies of Opie and his colleagues on family contagion in tuberculosis led to an exact understanding of the manner of spread of tuberculosis in children and adults, and to a wide adoption of the method of family study and case finding on a contact basis in tuberculosis clinics throughout the country. Opie's influence has been profound in this respect in public health practice."

From 1932 to 1941, while Professor of Pathology at Cornell Medical College, Opie continued to study tuberculosis and its spread in families. While at Peking he made an investigation of tuberculosis of the Chinese.

In 1941 as a specially invited guest of the Rockefeller Institute, Opie began to study the influence of diet on the occurrence of tumors of the liver produced in white rats by butter yellow. He also studied the pathogenesis of tumors of the liver and the role of ribonucleic acid in pancancerous lesions of the liver. In 1947 he studied and reported on the normal structure and degenerative changes of the cytoplasm of tumor cells.

Opie has interested himself more recently in the osmotic relationships between cells and intercellular fluid and has shown that the parenchymatous cells of liver or kidney or muscle fibers maintain by their metabolism an osmotic pressure approximately twice that of serum. He has demonstrated that with disturbed protein metabolism pathological changes occur in hepatic cells such as necrosis, hydrosis and lipidosis. These results have been reported each year from 1941 through 1957 in the *Journal of Experimental Medicine.*

Dr. Opie's academic honors include a Doctor of Science from Yale University in 1931, a Doctor of Laws from Washington University in 1940 and a Doctor of Laws from Johns Hopkins in 1947. Among the recognitions Dr. Opie has received for his scientific work is the Banting medal which was awarded to him by the American Diabetes Association in Toronto in 1946.

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BOOK REVIEWS

FOOD, NUTRITION AND DIET THERAPY. *By Marie V. Krause, B.S., M.S. Second edition, $6.00, pp. 621, 144 illus., W. B. Saunders Company, Philadelphia, 1952.*

This book is written primarily for the student nurse, with emphasis on the relation of diet to total nursing care. It is also aimed toward workers in the socio-economic field.

Part I concerns subjects of a basic nature. There are sections dealing with the metabolism of foods, general nutritional requirements, and a particularly interesting section discussing food habits of different races. There are valuable sections on nutrition and public health, especially dealing with problems of food handling and intoxication.

Part II deals with diet therapy. The normal hospital diet is described and emphasis placed on the proper approach to make meals attractive to the patient. There are sections dealing with systemic disease and surgical conditions. Brief descriptions are given of the physiology and medical treatment of the disorders concerned. However, the material is somewhat outdated in several instances. For example, in adrenal failure, therapy with DOCA is discussed, but no comments are given about steroid preparations. Diabetes mellitus is taken up in Chapter 24. Benedict's qualitative test for urinary sugar is given instead of the Clinitest method. The dietary system advocated for diabetics is almost wholly that published by the American Diabetes Association and the DIABETES GUIDE BOOK FOR THE PHYSICIAN of 1950. The exchange lists are presented, and there is a discussion of meal planning for correlation with insulin administration. However, the insulins discussed are regular and Protamine, or combinations thereof, and no mention is made of meal planning with the intermediate-acting insulins. Part III gives the dietary management of pregnancy, lactation and childhood.

The monograph is amply fortified with photographs and tables. There is a large section containing recipes and food tables. The book is, in general, very complete and is written for the person who is not a dietitian. A good bibliography is listed at the end of each section for further reading. There are also exercises given to stimulate study by the nurse or other student of dietetics.

ABSTRACTS

Azérad, E.; Ghata, J.; and Lewin, J. (Hôpital Beaujon, Paris, France): A STUDY ON DIABETIC COMA: MODIFICATIONS IN PROTEINS, LIPOPROTEINS AND GLUCOPROTEINS IN THE COURSE OF DIABETIC ACIDOKETOSIS. Presse méd. *66*:995-98, June 4, 1958.

In cases of severe untreated acidoketosis the lipoproteinogram is characterized by diminution of α lipoproteins. In nine out of eleven lactescent sera, marked increase of β lipoproteins is observed. Treatment produces an increase of the former and a decrease of the latter. The significance of the above modifications is discussed. (French)

Barta, L.; and Bedo, M. (First Dept. of Pediatrics, Med. Univ., and Institute of Nutrition, Budapest): INFLUENCE OF THE TEMPERATURE OF INGESTED SUGAR SOLUTIONS ON THE BLOOD-SUGAR LEVEL. Lancet *1*:1283, June 14, 1958.

The authors refer to a rarely mentioned and interesting difference between the action of cold and warm foods. (Bicknell, F., *Lancet*, 1957, ii, 388.) After eating cold foods hypoglycemia develops sooner than after ingestion of warm foods. In those diabetic patients who received their insulin before a supper consisting of cold meat, hypoglycemia developed sooner than in patients whose supper consisted of warm meat of the same quality and quantity. It was therefore decided to examine the difference between the effect of warm and cold foods upon the blood sugar level in a group of twelve male albino rats. The blood sugar rise was about 68 per cent greater after the administration of warm glucose solution than after the ingestion of cold glucose solution. This phenomenon is of importance in the dietetic treatment of diabetic patients. The consumption of a cold cream rich in sugar and protein causes a uniform and slow rise in blood sugar level; and this effect might be enhanced by the action of proteins in increasing the blood sugar.

Bergen, Stanley S.; Hilton, James G.; and Norton, William S. (Dept. of Medicine, St. Luke's Hosp., New York, N.Y.): EFFECT OF PHENETHYLBIGUANIDE ON ADRENAL FUNCTION AND RESPONSIVENESS AS MEASURED BY ACTH TEST. Proc. Soc. Exper. Biol. & Med. *98*:625-27, July 1958.

The authors were unable to demonstrate any alteration of baseline serum levels of seventeen hydroxycorticoids, nor any denation from normal in the responsiveness to a six-hour ACTH drip in six patients who were being treated with DBI. It is concluded that the hypoglycemic action of DBI is not mediated by alterations of adrenal secretion.

Bing, Richard J. (Washington University Sch. of Medicine, St. Louis, Mo.): METABOLISM OF THE HEART. Presse méd. *66*:1015-17, June 7, 1958.

Certain metabolic phases of the arrested, fibrillating and perfused heart have been studied by the author, as well as the metabolism of the human heart in situ. A description of some of the properties of the contractile proteins of the cardiac muscle is also given.

Birnbaum, David; and Kleeberg, Julius (Med. Dept. A, Hadassah University Hosp., Jerusalem, Israel): CARCINOMA OF THE PANCREAS: A CLINICAL STUDY BASED ON 84 CASES. Ann. Int. Med. *48*:1171-84, June 1958.

The clinical and pathological data of eighty-four cases of cancer of the pancreas were reviewed. Fasting blood sugar determinations were performed in sixty-three and twenty-nine (46 per cent) were found higher than normal. The incidence of hyperglycemia was the same among those with carcinoma in the head of the pancreas as among those in the body and tail. Seven additional patients with normal fasting values showed an abnormal glucose tolerance test.

Bloomfield, Arthur L. (Lane Medical Library, San Francisco, Calif.): A BIBLIOGRAPHY OF INTERNAL MEDICINE: DIABETES MELLITUS—FROM ROLLO (1798) TO BANTING (1921). A.M.A. Arch. Int. Med. *101*:1159-71, June 1958.

This is from the section "Evolution of Modern Medicine" which has as its purpose a review of the contributions of medical investigators and observers of the past to the present-day concepts of disease. The most outstanding contributions of investigators of diabetes mellitus are selected and well summarized.

Bonessa, C.; Mars, G.; and Morpurgo, A. (Institute of Medical Hydrology, University of Milan, Italy): ABOUT THE SIGNIFICANCE OF ELECTROMECHANICAL AND HEMODYNAMIC ALTERATIONS AND OF PLASMA ELECTROLYTE MODIFICATIONS DUE TO HYPOGLYCEMIA IN DIABETICS WITH CARDIO-ANGIOSCLEROSIS AND HYPERTENSION. Schweiz. med. Wchnschr. *87*: 1392-97, Nov. 16, 1957.

Studies carried out to elucidate the cardiac effects of insulin hypoglycemia in animals and man have given discordant results. The authors have studied the behavior of the electrical and mechanical activity of the heart, of hemodynamics and of plasma electrolytes before, during and after hypoglycemic coma induced in eleven diabetics over sixty years of age. These patients suffered from arteriosclerotic and/or hypertensive heart diseases, and had occasionally shown typical hypoglycemic phenomena during insulin therapy, without demonstrable consequences. The striking modifications of cardiac electrical activity caused by hypoglycemic coma, consisting mainly in an increased excitability and in a delayed repolarization, were found to be rapidly reversible in all cases. Variations in cardiac mechanical activity and in hemodynamics appeared, instead, to be less constant and by no means uniform; the average values obtained before, during and after hypoglycemic coma do not differ much. The appearance of an energetic-dynamic insufficiency, in Hegglin's sense, was never observed. Of the plasma electrolytes, only potassium showed a significant decrease. No patient developed symptoms or signs of cardiac or coronary insufficiency, during or after the insulin-induced coma. (French)

Bradley, T. R. (Dept. of Physiol., University of Melbourne, Melbourne, Australia): THE EFFECT OF INSULIN ON THE METABOLISM OF MAMMARY GLAND SLICES FROM HYPOPHYSECTOMIZED RATS. Austral. J. Exper. Biol. & M. Sc. *36*:183-90, April 1958.

Hypophysectomy of lactating rats resulted in severely de-

pressed in vitro glucose uptake by mammary gland slices. The glucose taken up by the slices appeared as lactic acid in the incubation medium. A decrease in sensitivity to insulin occurred within twenty-four hours; at forty-eight hours after the operation, insulin was virtually ineffective with respect to glucose uptake by such slices.

Brown, Meyer; Bernsohn, Joseph; and Finkle, John (Northwestern University Med. Sch., Chicago, Ill.; and Veterans Administration Hosp., Hines, Ill.): REGULATION OF CARBOHYDRATE METABOLISM IN SPINAL CORD INJURIES. Neurology *8*:547-50, July 1958.

Patients with injuries to the spinal cord in the cervical region show a significantly decreased tolerance to glucose in the oral glucose tolerance curve when compared to patients with dorsal and lumbar injuries. Adrenalin injected intravenously produces a significantly greater hyperglycemic response in patients with injuries to the dorsolumbar part of the spinal cord than in patients with injuries to the cervical spinal cord.

Bryson, William (Spring Grove State Hosp., Baltimore, Md.): CLINICAL TRIAL OF N-4-METHYL-BENZOLSULFONYL-N-BUTYLUREA (ORINASE) IN ADULT DIABETICS. Maryland M. J. 7:286-91, June 1958.

This report concerns the clinical effects of a hypoglycemic agent used as a substitute for insulin in the control, for a three-month period, of the diabetic syndrome in fifteen patients. Orinase proved to be the equivalent to or better than insulin in these fifteen elderly diabetics treated over a three-month period.

Christensen, S.; Dollerup, E.; Jensen, S. Eskjaer (Kommunehospitalet, Aarhus, Denmark): IDIOPATHIC HYPERLIPAEMIA, LATENT DIABETES MELLITUS AND SEVERE NEUROPATHY. Acta med. scandinav. *161*:57-68, May 1958.

A case of hyperlipemia, latent diabetes mellitus, intermittent claudication and severe neuropathy in a woman, aged fifty-eight, is reported. In the light of our present-day knowledge this case must be classified as one of idiopathic hyperlipemia associated with a diabetic metabolic abnormality. The neuropathy and the occlusive vascular disease of the lower limbs were not referable to long-term diabetes, but must be considered to be a sequela of the hyperlipemia.

Colbert, R. M.; Calton, F. M.; Dinda, Ruth E.; and Davies, Jack (Dept. of Anat., Washington University Med. Sch., St. Louis, Mo.): COMPETITIVE TRANSFER OF SORBOSE AND GLUCOSE IN PLACENTA OF RABBIT. Proc. Soc. Exper. Biol. & Med. 97:867-69, April 1958.

The authors infused sorbose alone and glucose plus sorbose intravenously into pregnant rabbits near term and demonstrated that there is a significant depression of permeability for sorbose when the maternal blood is overloaded with glucose.

Collins, John M.; and Seglenieks, Z. (Northfield Mental Hosp., Adelaide, Australia): GLUCAGON IN INSULIN THERAPY. M. J. Australia *1*:361-62, March 15, 1958.

The blood sugar levels of a patient in insulin coma were taken ten, twenty and forty minutes after interruption of coma with 4 mg. of glucagon given intravenously, 300 gm. of sucrose given by gavage, and 50 ml. of 50 per cent glucose solution given intravenously. The absence of both local and general untoward effects from glucagon is noted. Four milligrams of crystalline glucagon given intravenously proved a satisfactory method of terminating the insulin comas induced in this man. However, sucrose given by gavage was found just as satisfactory.

Colwell, Arthur R., Sr.; and Colwell, John A. (Dept. of Med., Northwestern Univ. Med. School, Chicago, Ill.): CLINICAL USE OF ORAL SULFONYLUREA COMPOUNDS IN DIABETES MELLITUS. Quart. Bull. Northwestern Univ. M. School *31*: 102-13, Summer 1957.

An excellent review of the subject of the sulfonylurea compounds as of April 1957.

Cornblath, Marvin; Levin, Ephraim Y.; and Marquetti, Estela (Dept. of Pediat., Sinai Hosp. of Baltimore and Johns Hopkins Med. Institutions, Baltimore, Md.): STUDIES OF CARBOHYDRATE METABOLISM IN THE NEWBORN. II. THE EFFECT OF GLUCAGON ON THE CONCENTRATION OF SUGAR IN CAPILLARY BLOOD OF THE NEWBORN INFANT. Pediatrics *21*: 885-92, June 1958.

Glucagon was administered intramuscularly in three to fifteen hours after birth to eight full-term infants, and by the intravenous route within thirty-one hours of birth to ten other full-term infants. A rise in blood sugar in one hour occurred in both with a mean increase of 32 mg. per cent in the former and 50 mg. per cent in the latter, though the intravenous route produced a more prolonged hyperglycemia. Ten premature infants given 50 mg./kg. I.V. within thirty hours of birth had a less definite rise in blood sugar than in the mature born, and the response was similar at age five days or older. Mature babies of five to fourteen days responded to glucagon with a hyperglycemic peak at thirty to forty-five minutes and a return to fasting in sixty to ninety minutes which is more like the response of the older child and adult.

Corvilain, J.; Tagnon, R.; and Scoby, L. (Clin. Méd., Hôp. Saint-Pierre and Lab. de Médicine Expérimentale, Université Libre de Bruxelles, Brussels, Belgium): INFLUENCE OF BZ-55 ON THE TRANSFORMATION TO GLUCOSE OF INJECTED FRUCTOSE IN THE DIABETIC. Rev. franç. études clin. et biol. *3*:613-15, June 1958.

The hyperglycemia induced in nine diabetic patients by intravenous fructose has been found to be considerably diminished by giving carbutamide, this substance having no action on the rate of uptake of fructose by the tissues or the concentration of fructose in the blood. (French)

Csaky, T. Z.; and Glenn, J. E. (Dept. of Pharmacol., University of North Carolina Sch. of Medicine, Chapel Hill, N. C.): DISTRIBUTION OF 3-METHYLGLUCOSE IN ORGANS OF RAT. Proc. Soc. Exper. Biol. & Med. 98:400-01, June 1958.

The authors report the relative permeability of 3-methylglucose in various organs of the rat. This substance has been shown to be excreted quantitatively within a period of twenty-four hours in the urine without any metabolic change. It is demonstrated that volume of distribution of this substance in rats is 35 to 37 per cent of body weight. The liver and myocardium contain about the same concentration of 3-methylglucose as plasma water; whereas brain, gastrointestinal tract, and leg muscle contain approximately one third to one fourth as much.

Danowski, Thaddeus S.; Greenman, Lawrence; Weigand, Franklin A.; and Mateer, Frank M. (Dept. of Res. Medicine, University of Pittsburgh, the Renziehausen Memorial Ward and The Children's Hospital of Pittsburgh, Pa.): ACIDOSIS AND

COMA IN JUVENILE DIABETICS. A.M.A. J. Dis. Child. 93:341-56, April 1957.

This is a review of a series of 191 instances of diabetic acidosis or coma in a group of 422 juvenile diabetics collected over the past nine years to see if deaths in this group are preventable. Mortality in the series was below 3 per cent.

The events preceding the acidosis were infection in 61 per cent of the entire group; uncontrolled glycosuria (42 per cent); insulin omission (20 per cent); emotional upsets (in one of five). Thirty-three per cent were previously unknown diabetics.

The commonest symptoms and signs were tachypnea, vomiting, Kussmaul breathing, stupor, abdominal pain and coma (in that order).

Therapy included regular insulin averaging 25-50 units initially in the 0- to eight-year-old and 50-100 units initially in the older child. Fluids to replace the water and electrolyte deficit were given usually as nonalkalinizing solutions unless the CO_2 was below 7 mEq./L. Glucose solution was used when the blood sugar was falling, or when the initial glucose level was below 300 mg. per cent, and was never given initially without electrolytes. K as buffered PO_4 was given at about the fourth hour of treatment after voiding well, usually as 40 mEq./L. in 5 per cent glucose. Blood levels of K were determined. The usual estimated deficit of K in coma was 5-6 mEq./kg. body weight. Dextran or plasma or blood was given to those in shock.

In conclusion, it was stated that since the deaths in this series occurred in unrecognized diabetics only, therefore the mortality in comatose patients could be reduced only by recognizing the disease earlier and treating it effectively.

Darwish, A. E.; Fikry, M. Essam (Dept. of Medicine, Alexandria University, Egypt): THE ROLE OF VITAMIN B_{12} IN CARBOHYDRATE METABOLISM. Lancet 1:1388, June 28, 1958.

The authors suggest that vitamin B_{12} is concerned in carbohydrate metabolism, probably through the synthesis of glutathione, which is found in reduced amounts in the red cells in pernicious anemia in rats deficient in vitamin B_{12}. These rats were also less able to dissimilate glucose to ribose than normal rats. The importance of ribose in carbohydrate metabolism is discussed. The authors studied the role of vitamin B_{12} in carbohydrate metabolism both in normal people and in diabetic patients. Fifteen of twenty subjects became more sensitive to insulin after receiving B_{12} in a dose of 1,000 μg. daily for two days. Four out of five patients developed a flatter intravenous glucose-tolerance curve after the addition of 1,000 μg. of vitamin B_{12} intravenously. Five diabetic patients and three normal people were made hyperglycemic by prednisone. The glucose-tolerance curves of these patients became more normal on B_{12}, and the subjects became more sensitive to insulin. The authors discussed possible explanations for the above results.

Daughaday, William H. (Washington University Sch. of Med. and Barnes Hosp., St. Louis, Mo.): DIETARY TREATMENT OF ADULTS WITH DIABETES MELLITUS. J.A.M.A. 167:859-62, June 1958.

This is a report to the Council on Foods and Nutrition of the American Medical Association and the article is part of a nutrition education program which is assisted by the Nutrition Foundation, Inc.

The control of dietary carbohydrate and caloric intake continues to be an important element in the treatment of patients with diabetes mellitus. The composition of such diets no longer differs greatly from the diets eaten by the nondiabetic population. The ability to distribute the daily carbohydrate intake in different ways permits the physician to overcome the deficiencies of exogenous insulin preparations. The technics of dietary education have been greatly simplified, and educational material is now available that should improve both hospital and office treatment of this disease.

De Groot, C. A.; and Weber, J. F. (Pharmacological Dept., University of Leiden, Leiden, Netherlands): INFLUENCE OF INSULIN AND HYPOPHYSIS ON LIVER GLYCOGEN. Acta physiol. et pharmacol. neerl. 7:93-105, March 1958.

In adrenal-demedullated, alloxan-diabetic, hypophysectomized rats an insulin injection of 0.25 U did not increase liver glycogen after two hours in spite of a glucose load; nonetheless, a glycogen retention seems to be involved since insulin hypoglycemia is not accompanied by increased glucose output by the liver. If the hypophysis is left intact the only apparent difference is the increase in blood sugar levels after a glucose load which now becomes significant.

De Vaughn, N. H. (Augusta, Ga.): THE HEART IN DIABETIC KETOSIS. J.M.A. Georgia 47:361-62, July 1958.

Shock in diabetic ketosis may produce such a decrease in coronary blood flow that myocardial ischemia and heart failure result. This circulatory stagnation and the increased viscosity of the blood due to dehydration predispose to coronary thrombosis in atherosclerotic diabetics. Changes in ionic concentration also disturb the function of the heart. Potassium is the element whose fluctuation is the most important in this respect. Both deficiency and excess of potassium cause characteristic, but not specific, electrocardiographic changes. The principal danger of hyperkalemia is ventricular fibrillation. Hypokalemia causes heart failure with central shock as its chief terminal manifestation.

De Venanzi, F. (Instituto de Investigaciones Médicas, Fundación Luis Roche, Caracas, Venezuela): EFFECT OF SOME MONOSACCHARIDES ON SERUM INORGANIC PHOSPHATE IN THE NORMAL DOG. Proc. Soc. Exper. Biol. & Med. 96:530-32, November 1957.

The author demonstrates that D-glucose, L-sorbose, and D-sorbitol produce a definite decrease and D-arabinose a definite rise in serum phosphorus after intravenous administration of the above-listed monosaccharides. D-galactose, L-rhamnose, and D-xylose have slight reducing effect on serum phosphorus. D-fructose produces a slight increase at forty-five-minutes. This effect seems to be superficially related to the arrangement of the OH group in carbons 3 and 4 in the hexose molecule.

Ditzel, Jørn; and Camerini-Davalos, Rafael (Baker Clin. Res. Lab., New England Deaconess Hosp. and Joslin Clin., Boston, Mass.): REVERSIBILITY OF VENULAR DILATATION AND CONGESTION IN DIABETIC SUBJECTS OVER A PERIOD OF HOURS. Proc. Soc. Exper. Biol. & Med. 97:475-77, February 1958.

Venular dilatation and congestion, as determined by the use of the stereoscopic microscope on the bulbar conjunctiva, was performed on fifteen diabetic subjects. The patients were photographed from two to five times at irregular intervals between 8 a.m. and 6 p.m., and the vascular changes were evaluated

and graded by measurements from the enlarged photomicrographs. The authors demonstrate that venular dilatation and congestion observed in the conjunctiva of diabetics may fluctuate widely within a period of hours. Venular dilatation was usually least marked in the later afternoon, at the time when the insulin effect of the isophane insulin taken by these patients was maximum. No statistical data are presented to support this correlation.

Dodgen, Charles L.; and Muntwyler, Edward (Dept. of Biochemistry, State University of New York, Downstate Med. Center, Brooklyn, N. Y.): LIVER GLYCOGEN IN POTASSIUM-DEFICIENT RATS FOLLOWING CARBOHYDRATE AND ALANINE ADMINISTRATION, WITH AND WITHOUT POTASSIUM. Proc. Soc. Exper. Biol. & Med. *98*:402-04, June 1958.

No changes in liver glycogen, water and potassium were found in potassium deficient rats as compared to normal, following the administration of glucose, fructose, or alanine, with or without potassium.

Dreiling, David A.; Janowitz, Henry D.; Haemmerli, U. Peter; and Marshall, David (Depts. of Surgery and Medicine, The Mount Sinai Hosp., New York, N. Y.): THE EFFECT OF GLUCAGON ON THE EXOCRINE PANCREATIC SECRETION OF MAN. J. Mt. Sinai Hosp., New York *25*:240-43, May-June 1958.

Glucagon-induced hyperglycemia did not affect the rate of flow, bicarbonate secretion, nor the rate of enzyme secretion by the pancreas in patients with and without pancreatic inflammation. Glucagon-induced hyperglycemia is accompanied by a reciprocal depression of the blood amylase, an effect apparently extrapancreatic in locus of action, and probably secondary to an induced alteration in rate of glucose utilization.

Ellison, R. J.; Wilson, W. P.; and Weiss, E. B. (Dept. of Psychiatry, Duke University Sch. of Med., and Vet. Admin. Hosp., Durham, N. C.): CHANGES IN CEREBRAL POTASSIUM DURING HYPOGLYCEMIA. Proc. Soc. Exper. Biol. & Med. *98*: 128-29, May 1958.

The authors produced severe hypoglycemia by the intravenous injection of insulin in twelve rats. Analysis of the brain revealed no difference in water content in the insulin treated as opposed to the control rats. However, there was a statistically significant difference in the cerebral potassium levels of the dried brain of rats that were treated with insulin as opposed to the controls. It seems difficult to interpret these findings, although the authors suggest that insulin does result in increased cerebral glucose utilization.

Elrick, H.; Arai, Y.; and Hlad, C. J., Jr. (Radioisotope Serv., Veterans Administration Hosp., and Dept. of Med., Univ. of Colorado Sch. of Medicine, Denver, Colo.): THE ACTION OF GLUCAGON-INSULIN MIXTURES IN DIABETIC PATIENTS. J. Clin. Endocrinol. *18*:825-33, August 1958.

The observations reported here indicate that in the diabetic patient glucagon and insulin together increase the specific rate constant for peripheral glucose utilization significantly more than does insulin alone. The response of arterial glucose levels to a combined glucagon-insulin infusion appears to be influenced primarily by the fasting blood glucose concentration, and secondarily by the ratio of glucagon to insulin in the infusion.

Since glucagon is most effective in stabilizing blood sugar concentration and increasing peripheral glucose uptake when the blood sugar level is above 170 mg. per 100 ml., it might prove useful in the treatment of impending or actual diabetic acidosis. Glucagon is most likely to be of value in the treatment of acute derangements of diabetic control, whether they be hyperglycemic or hypoglycemic in nature.

Elrick, H.; Huffman, E. R.; Hlad, C. J., Jr.; Whipple, N.; and Staub, A. (Radioisotope and Med. Servs., Veterans Administration Hosp., and Dept. of Med., Univ. of Colorado Sch. of Medicine, Denver, Colo.): EFFECTS OF GLUCAGON ON RENAL FUNCTION IN MAN. J. Clin. Endocrinol. *18*:813-24, August 1958.

The data presented show that glucagon causes a significant and sustained increase in the renal clearance ratio of inorganic phosphorus, and a significant but transient increase in the renal clearance ratios of sodium, chloride and potassium. These effects were also significantly different from those observed with glucose despite the fact that the magnitudes of hyperglycemia induced by these two substances were not significantly different. Because glucagon markedly increases the excretion of electrolytes but does not change the filtered load appreciably, it would appear to have an action on the renal tubule. In view of the questionable significance of the changes in glomerular filtration rate and renal plasma flow, the possibility of an action of glucagon on the glomerulus cannot be ruled out.

The mechanism of these renal effects is unknown.

Elrick, Harold; Konigsberg, I. R.; and Arai, Y. (Radioisotope Serv., V. A. Hosp., and Depts. of Medicine and Pediatrics, University of Colorado Sch. of Med., Denver, Colo.): EFFECT OF GLUCAGON ON GROWTH OF CHICK EMBRYO. Proc. Soc. Exper. Biol. & Med. 97:542-44, March 1958.

It has been previously reported that the administration of glucagon caused a significant increase in the weight of chick embryos, which suggested that glucagon had growth stimulating potentialities. The previous preparations were relatively crude preparations of glucagon. These studies were repeated using purified crystalline glucagon. No significant effect on the weight of embryos was observed.

Fasella, Paolo; Baglioni, Corrado; Turano, Carlo; and Siliprandi, Noris (Inst. of Biological Chemistry in the University of Camerino, Italy): ACTION OF CITRATE AND OXALACETATE ON DIETARY AND DIABETIC KETOSIS. Lancet *1*:1097, May 24, 1958.

The authors examine the action of citrate and oxalacetate on experimental dietary and diabetic ketosis in the rat and the dog. Results of these experiments show that dietary ketonuria is practically abolished by intraperitoneal injection of either citrate or oxalacetate. Diabetic ketosis, on the other hand, is not significantly influenced either by citrate or by oxalacetate injected intravenously. The discrepancy between the results obtained in diabetic and in dietary ketosis suggests that the pathogenic mechanisms responsible for these two conditions are fundamentally different. Diabetic ketosis may not be attributable, as dietary ketosis is, to a deficiency of oxalacetate or of citrate but rather, to a disturbance in the Krebs cycle.

Fulton, Richard L.; and Bell, George E. (Ohio State University and Columbus Med. Center, Columbus, Ohio): MANAGEMENT OF DIABETES WITH TOLBUTAMIDE (ORINASE). Ohio M. J. 53:1288-91, November 1957.

There is no definite correlation between the response of a patient to the tolbutamide test and the possibility of his being

maintained on tolbutamide therapy. Tolbutamide does control a significant number of diabetic patients. It would probably be worth while to offer a trial of therapy with tolbutamide to every adult diabetic. The authors have already adopted this policy with their diabetic patients.

Gastineau, Clifford F. (Rochester, Minnesota): DIABETIC ACIDOSIS: SOME PRINCIPLES OF TREATMENT. J.M.A. Georgia 47:183-85, April 1958.

Patients with acidosis of moderate severity will survive if treated by any of several methods, but those with increasingly severe acidosis seem to approach a point of irreversibility beyond which no amount of knowledge will bring successful results. As the severity of the acidosis approaches this point, the margin of error permitted the physician becomes less and less; and in severe acidosis the treatment must be adapted to the needs of the moment with a maximum of precision.

Gelin, A.; and Pirart, J. (Brussels, Belgium): CRANIAL TRAUMA FOLLOWED BY ACUTE PANCREATITIS AND ACUTE DIABETES (COMA): CURE OF THE PANCREATITIS AND REMISSION OF DIABETES. Acta gastro-enterol. belg. 20:724-33, August-September 1957.

A sixty-year-old female, without a family history of diabetes, developed acute pancreatitis and diabetes four days after cranial and thoracic injury.

The relationship of trauma, acute pancreatitis and diabetes is discussed. The authors conclude that with close supervision, sufficient parenteral feedings and early insulin therapy, it is possible to prevent the complications of diabetes occurring after acute pancreatitis. (French)

Gillman, J.; Gilbert, C.; Epstein, E.; and Allan, J. C. (University of the Witwatersrand, Johannesburg, South Africa): KETOGENIC ACTION OF HUMAN ANTERIOR PITUITARY IN DIABETIC BABOONS. Lancet 1:1226, June 7, 1958.

The authors were interested in the ketogenic effect of human growth hormone in normal, and in diabetic, hypophysectomized subjects. They studied various endocrine factors controlling the lipemia (including cholesterolemia), ketonemia, and hyperglycemia of pancreatectomized, insulin-deprived baboons. Removal of the hypophysis from pancreatectomized baboons abolishes the severe lipemia and prevents the ketosis but does not affect the hyperglycemia. The administration of cortisone to pancreatectomized-hypophysectomized baboons results in the development of a lipemia even more severe than that in the pancreatectomized baboon before hypophysectomy, but is not associated with a ketosis. The administration of acetone-dried human anterior pituitary to pancreatectomized-hypophysectomized baboons intensifies the hyperglycemia and results in severe ketosis unaccompanied by lipemia. The complete diabetic picture in the pancreatectomized-hypophysectomized baboon can be produced only by the administration of cortisone in conjunction with crude pituitary extract. Crude human anterior pituitary produces severe toxic effects, leading to a high mortality in pancreatectomized-hypophysectomized baboons deprived of insulin. In these circumstances the insulin requirements of the baboon are greatly increased. The administration of twice the amount of crude human anterior pituitary to normal baboons failed to affect the blood sugar or to produce a ketosis. It is thus apparent that in the pancreatectomized baboon the lipemia depends on the degree of functional activity of the adrenal cortex, and the severity of the ketosis is related to the presence of some factor in the anterior lobe of the pituitary and is not determined by the gravity of the lipemia. The studies of the authors show that human anterior pituitary is ketogenic for the pancreatectomized-hypophysectomized baboon no less than it is for the hypophysectomized human diabetic patient.

Hackedorn, Howard M. (Seattle, Washington): TOLBUTAMIDE COMPARED TO INSULIN FOR DIABETIC CONTROL. Northwest Med. 57:749-50, June 1958.

Tolbutamide does not facilitate glucose utilization. It does not directly influence other hormones except the hypoglycemic effect of insulin, nor do other hormones affect it. It is not an insulin substitute and one would wish to determine its ultimate value in diabetic management, because disturbances in fat and protein metabolism, as well as carbohydrate metabolism, are significant defects of diabetes. Clinically we measure only hyperglycemia and ketosis, yet we know both protein and fat metabolism are profoundly affected by insulin and lack of it. While tolbutamide is controlling the hyperglycemia, one wonders if there is simultaneously an impairment of the tissue fat and protein metabolism that will become manifest after prolonged therapy.

It is possible that the control of hyperglycemia alone might be a screen that prevents effective observation of more profound tissue changes occurring in the continued absence of adequate insulin supplies. Therefore these patients treated with tolbutamide alone should continue to have regular and careful surveys with these possibilities clearly in mind.

Hall, G. H.; Crowley, Mary F.; and Bloom, Arnold (Whittington Hosp., London, England): ORAL TREATMENT OF DIABETES. TRIAL OF PHENETHYLDIGUANIDE (DBI). Brit. M. J. 2:71-74, July 12, 1958.

The authors report the administration of a new oral hypoglycemic substance, phenethyldiguanide (DBI), to forty diabetic patients of all grades of severity. A significant blood sugar fall occurred in about two thirds of the cases. Ketosis was not rectified and occasionally developed in patients on DBI. In five cases in which tolbutamide proved ineffective, DBI produced a fall in blood sugar. Ill effects (usually nausea, vomiting or diarrhea) appeared in twenty-five of the forty cases, precluding the general use of DBI in diabetic therapy.

Halmi, N. S.; Hass, A. C.; Widner, R. R.; and Spirtos, B. N. (Dept. of Anat., State University of Iowa, Iowa City, Ia.): EFFECT OF FASTING OR UNDERFEEDING ON GLUCOSE TOLERANCE OF RATS. Proc. Soc. Exper. Biol. & Med. 96:839-42, December 1957.

The generally accepted concept that fasting for several days results in a glucose tolerance of the diabetic type has just recently been questioned in humans. These authors present data that make it difficult to accept this concept in rats as well. Rats fasted from one to six days failed to show a decrease of oral glucose tolerance. The return of blood sugar levels to normal after intravenous administration of glucose was somewhat delayed after one to six days of fasting. The delay was not proportional to the duration of the fast. Chronically underfed rats demonstrated an increase tolerance to glucose. In no instance was there any marked deficiency in hepatic glycogen concentration.

Heinsius, E. (Dept. of Ophthalmology, Heidberg Hosp., Hamburg, Germany): DIABETIC RETINITIS IN CHILDHOOD. German M. Month. *2*:183-84, June 1957.

On the basis of the author's observations, it appears essential that every child with diabetes undergo an ophthalmoscopic examination at least once every four years.* It is obvious that in all cases with retinal changes early therapeutic measures are indicated. As the prognosis in diabetic retinitis is doubtful, the attending physician should advise the parents about the child's choice of profession.

*(*Editor's note*: This seems grossly inadequate for careful control and early recognition of retinal changes.)

Hoenig, V.; Schück, O.; Fischer, O.; Hoenigová, J.; and Patová, V. (Ie Clinique Méd. de l'Université Charles IV, Prague, Czechoslovakia): HYPERPHOSPHATURIE APRES ADMINISTRATION INTRAVEINEUSE DE GLUCOSE ET DE FRUCTOSE. Acta med. scandinav. *161*:79-84, May 16, 1958.

The renal excretion of inorganic phosphorus following intravenous administration of 25 gm. of glucose or fructose (40 per cent solution) was studied in forty subjects (twenty controls and twenty cirrhotics). There was a more marked rise in the average urinary phosphorus following fructose than following glucose both in the cirrhotic and in the control group. The average rises in the cirrhotic and in the control group did not differ. It was shown that following fructose the tubular reabsorption of phosphorus is more depressed than following glucose. (French)

John, Henry J. (University, St. Luke's, and Huron Road Hosps., Cleveland, Ohio): INFORMATION AND MISINFORMATION GAINED FROM FASTING BLOOD SUGAR ALONE IN DIABETES THERAPY. Ohio M. J. *53*:1284-87, November 1957.

One can be misled if only the fasting blood sugar is done and no attention paid as to what happens the rest of the day. One cannot have too much laboratory information on a patient, or, to put it in other words, the lack of adequate information leads often to misconception of the real status of the patient's diabetic state, namely, his proper control on any given routine.

Johnson, Einer W., Jr. (Sect. of Orthopedic Surgery, Mayo Clin. and Mayo Foundation, Rochester, Minn.): THE SURGICAL MANAGEMENT OF DIABETIC COMPLICATIONS. West Virginia M. J. *54*:157-64, May 1958.

The surgeon should be aware of the three phases of gangrene and he must be prepared to act expeditiously when the final phase presents itself. These three are, first, gangrene without secondary infection; second, gangrene with infection which is under control; and third, gangrene with infection which is not controlled and which appears to be leading to bacteremia and toxic psychosis.

Kalstone, Bernard M. (Post-Graduate Dept. of Med., Louisiana State University Sch. of Med., Shreveport, La.): DETAILS IN THE PREVENTION AND TREATMENT OF DIABETIC ACIDOSIS. Am. Pract. & Digest Treat. *9*:207-12, February 1958.

Diabetic acidosis almost always can be prevented by proper instruction of the patient. If it should occur, adequate insulin dosage early, rapid fluid and electrolyte repletion, glucose in six to eight hours and *constant* patient surveillance are indicated for its successful treatment.

Karasek, M. A.; Britton, Barbara; and Wick, Arne N. (Scripps Clin. and Res. Foundation, La Jolla, Calif.): EFFECT OF MODIFICATION OF INSULIN ON ITS DEGRADATION AND BIOLOGICAL ACTIVITY. Proc. Soc. Exper. Biol. & Med. *97*:242-46, January 1958.

Biologic activity of insulin was determined in eviscerated-nephrectomized female rabbits by the constant infusion of glucose. Utilization was calculated from the quantity of glucose infused. The administration of insulin results in a marked increase in the rate of glucose utilization. This preparation was also used to determine the I^{131} insulin degradation rate. Alteration of the relatively specific structural components of the insulin molecule was performed with nine different substances. Those reagents which interfered with the rate at which insulin was degraded also destroyed the biologic activity of insulin. The findings are significant in the problems of the essential functional units of the insulin molecule.

Kennedy, David J. (Syracuse, N. Y.): ACUTE ALVEOLAR ABSCESS AND MUMPS IN AN UNDETECTED DIABETIC. Oral Surg. *11*:461-63, May 1958.

A case history has been described in which a patient with a triad of diseases presented a difficult problem in diagnosis and management. Superimposed on a basic metabolic disease were separate viral and bacterial infections. Correlation of signs, symptoms, history and laboratory findings established a diagnosis of diabetes mellitus, mumps and alveolar abscess.

Koenig, Robert P. (University of Cincinnati Coll. of Med., Cincinnati, Ohio): MANAGEMENT OF PREGNANT DIABETICS. (A TEN-YEAR SURVEY OF THE RESULTS OF DIABETES COMPLICATED BY PREGNANCY IN TWO PRIVATE GENERAL HOSPITALS—53 CASES). Ohio M. J. *53*:1297-1301, November 1957.

Fifty-three cases of diabetic women who became pregnant are discussed. Care of the women differed in two hospitals. Obstetrician-internist team management is shown to be of value in the management of diabetes complicated by pregnancy by reducing complications of both pregnancy and of diabetes in the mother and by reducing the infant mortality. Maternal morbidity and mortality of diabetes complicated by pregnancy was shown to be controlled in these two series of cases. Details concerning diabetic care, the type of delivery and anesthesia and tubal ligations are presented.

Kotake, Yahito (Dept. of Biochemistry, Wakayama Med. Coll., Wakayama, Japan): EXPERIMENTS ON THE CHRONIC DIABETIC SYMPTOMS CAUSED BY XANTHURENIC ACID—AN ABNORMAL METABOLITE OF TRYPTOPHAN. Med. J. Osaka Univ. *8*:25-36, Supplement March 1958.

The author has conducted a series of experiments on the diabetic symptoms in albino rats caused by the administration of xanthurenic acid (XA), an abnormal metabolite of tryptophan. Evidence has been presented that XA is related to the development of human diabetes. Evidence also suggests that there is a close relationship between animal and human diabetes.

Krall, Leo P.; and Camerini-Davalos, Rafael (Joslin Clinic and New England Deaconess Hosp., Boston, Mass.): CLINICAL TRIALS WITH DBI, A NEW NONSULFONYLUREA ORAL HYPOGLYCEMIC AGENT. A.M.A. Arch. Int. Med. *102*:25-31, July 1958.

N′-β-phenethylformamidinyliminourea hydrochloride (DBI) is a new nonsulfonylurea compound with blood-sugar-lowering ability when given to patients with a restricted diet. The mechanism of action is not definitely known. It is a new

preparation, still under investigation, effective in milligram doses, which in spite of annoying side effects may have therapeutic usefulness in the future, providing that no toxic effects will be found.

One hundred and four patients were observed sufficiently to indicate that ninety (86 per cent) showed demonstrable blood-sugar-lowering effect (according to the criteria listed), although nineteen (18 per cent) were discontinued from the series because of side effects. Although the primary object of the study was blood-sugar lowering, "good" or "fair" control was achieved in sixty-six of the eighty-eight evaluated in this manner. Sixty patients are still maintained with DBI. Only six of these take supplemental insulin, and these take less than 50 per cent of their previous daily dose. Frequent liver function, blood, and other studies have shown no evidence of toxicity to date. There was an observable blood-sugar-lowering effect in every age group test and in every type of patient, including juvenile diabetics, in this series.

Lamy, M.; Frézal, J.; and de Grouchy, J. (Clin. de Génétique Médicale de la Faculté de Médicine, Hôp. des Enfants-Malades, Paris, France): RESULTATS D'UNE ENQUETE SUR L'HEREDITE DU DIABETE SUCRE. Rev. franç. études clin. et biol. *2*:907-19, November 1957.

The authors have expressed the familial incidence of diabetes in 500 established diabetics in relation to the age of onset of the disease; for the late diabetics, the type of diabetes, the weight and insulin dosage of the cases occurring in the families have been studied. No correlation has been found between maternal age or parity and the incidence of diabetes. The familial incidence was the same for primary and so-called secondary diabetes.

Six and fifty-nine hundredths per cent of the relatives were found to be diabetic. The incidence was higher in relatives of late diabetics, which can probably be explained by the greater age of the subjects. The incidence of diabetes in siblings was 3.1 per cent, approximately the same for both juvenile and late diabetics. Only in the severe, insulin-treated diabetics, whether juvenile or late, were the following found: consanguineous marriages in the parents; diabetes of both paternal and maternal relations; an increase in the sibling diabetes incidence if one parent was diabetic. These observations suggest that a mild adult diabetes is transmitted (in heterozygotes) by a single gene, which in homozygotes results in severe diabetes, whether juvenile or late. (French)

Lancaster, W. M.; and Murray, Ian (Dept. for Metabolic Diseases, Victoria Infirmary, Glasgow, Scotland): FURTHER EXPERIENCE WITH THE "LENTE" INSULINS. Brit. M. J. *1*:1331-33, June 7, 1958.

The authors review 335 diabetic patients treated for at least six months with insulin zinc suspensions (I.Z.S.). Satisfactory control was obtained in 82.5 per cent of 134 new patients and in 64.7 per cent of 201 patients previously receiving some other form of insulin. In ninety-four of the latter, I.Z.S. produced a better degree of control. The I.Z.S. proved unsatisfactory in fifteen patients. In the presence of intercurrent infections, lapse from diabetic control from I.Z.S. appears to occur more readily than with some other insulins. It is suggested that in such circumstances it is I.Z.S. crystalline which becomes relatively ineffective since transfer temporarily to two injections daily of I.Z.S. amorphous re-establishes control.

Landau, Bernard R.; Levine, Herbert J.; and Hertz, Roy (Harvard Med. Sch.; Massachusetts Genl. Hosp., Boston, Mass.; and National Cancer Inst., Bethesda, Md.): PROLONGED GLUCAGON ADMINISTRATION IN A CASE OF HYPERINSULINISM DUE TO DISSEMINATED ISLET-CELL CARCINOMA. New England J. Med. *259*:286-88, Aug. 7, 1958.

A twenty-eight-year-old woman with hyperinsulinism attributable to disseminated islet-cell carcinoma received glucagon continuously over a fifty-five-day period. Glucagon was found to have a hyperglycemic effect throughout this period, as determined by the hypoglycemic response of the patient during brief periods of glucagon withdrawal. Glucagon administration decreased the frequency of hypoglycemic attacks and the requirement for exogenous glucose. The mechanism of glucagon action in relation to these observations is discussed.

Lapresle, C.; and Grabar, P. (Serv. de Chimie Microbienne, Inst. Pasteur, Paris, France): COMPARATIVE STUDY OF BI-DIAZOTIZED BENZIDINE AND TANNIC ACID IN THE HEMAGGLUTINATION REACTIONS OBTAINED WITH INSULIN PREPARATIONS AND WITH SERUM OF DIABETICS TREATED WITH INSULIN. Rev. franç. études clin. et biol. *3*:57-58, January 1958.

The authors have made further studies on the antigenic impurities which are present in certain insulin preparations and may be responsible for positive hemagglutination reactions (Boyden's technic). They show that these antigenic fractions may produce the same agglutination phenomena when attached to red blood cells by bidiazotized benzidine.

Larsson, Yngve (Pediat. Clin. of Karolinska Inst., Kronprinsessan Lovisas Barnsjukhus, Stockholm, Sweden): THE ISLETS OF LANGERHANS IN PANCREATIC FIBROSIS. Pediatrics *21*:893-902, June 1958.

The author comments on the rare appearance of diabetes in cystic fibrosis of the pancreas, and undertakes a quantitative histologic study in nine rabbits in which the pancreatic duct was ligated. Fibrosis of the pancreas occurred in all of the animals with acinar atrophy, cystic dilatation of the ducts, between which numerous small islets of Langerhans of fetal type were compressed (a picture similar to that in cystic fibrosis of the pancreas and in the fetus). Quantitatively the islet volume was reduced in size but alpha-to-beta cell relations were normal. Eight of the nine animals had normal intravenous glucose tolerance curves, as did also three patients with known cystic fibrosis of the pancreas. Diabetes did not appear in the rabbits, and they were found to be alloxan resistant, just as is the newborn. The author suggests that the pancreatic duct epithelium has ambipotency in certain postnatal conditions just as it does in fetal life.

Leevy, Carroll M. (Hepatic-Metabolic Clin., Dept. of Medicine, Jersey City Med. Center and Seton Hall Coll. of Medicine, Jersey City, N. J.): THE LIVER IN DIABETES MELLITUS. J. M. Soc. New Jersey *55*:151-56, April 1958.

The liver, which is the metabolic center of the body, is always damaged in diabetes mellitus. Occasionally, liver disease may be responsible for a diabetic syndrome which will disappear with improvement in hepatic reserve. This may be due to disturbance of hepatic regulation of blood sugar or latent diabetes made manifest by liver injury. The prednisone-glucose tolerance test is helpful in determining which mechanism is responsible. Abnormality in carbohydrate, protein and fat metabolism due to an insulin insufficiency is accompanied by

a high incidence of liver dysfunction, fatty liver and nutritional cirrhosis. Hepatic changes should be prevented by adequate control of hyperglycemia and glycosuria and attention to general nutrition.

Lestradet, H.; Besse, J.; and Jezequel, C. (Paris, France): A STUDY ON THE ACTION OF HYPOGLYCEMIA-INDUCING SUBSTANCES IN A GROUP OF 275 CHILDREN. Presse méd. 65:553-56, March 23, 1957.

A study on the action of hypoglycemia-inducing substances was carried out among 275 children, ages three to seventeen, divided into seven groups. One of these comprised children receiving 2254 RP; another, subjects receiving D860; and one more, patients given placebos in the same dosage. Results evaluated on the basis of average amount of insulin required to keep glycosuria rates at 5 to 20 gm. for twenty-four hours demonstrated that insulinotherapy should so far be considered the basic treatment for diabetes mellitus in children.

Letchner, A. (Harrow-Weald, Middlesex, England): DIABETES MELLITUS. Brit. M. J. *1*:1418, June 14, 1958.

In a letter the author described an interesting case of diabetes mellitus which he saw in 1939, in which the presenting symptom was painful dysphagia.

Levine, Rachmiel (Dept. of Metabolic and Endocrine Res., and Dept. of Medicine of the Michael Reese Hosp., Chicago, Ill.): ETIOLOGIC FACTORS IN DIABETES MELLITUS IN MAN. Illinois M. J. *113*:286-88, June 1958.

No conclusive answers are presently available to the problem of the etiology of diabetes mellitus in man. Earlier the author had more confidence in genetic factors than today. A constitutional tendency to diabetes mellitus has been thought to be inherited as a recessive trait, but the frequency of the disease in the general population is too high to be explained by this factor alone or on the basis of a normal rate of mutation. A recent paper from Germany analyzed rats whose parents had been made diabetic with alloxan. The findings were as follows: (1) there was increased fetal mortality; (2) the young rats of alloxan-treated parents showed abnormal glucose tolerance curves; and (3) at four to six months, several rats exhibited spontaneous hyperglycemia. In another study, reported from Japan, alloxan-treated rabbits were mated and each succeeding generation of offspring was similarly given alloxan. The second generation rabbits required less alloxan to become diabetic, and the third and fourth generations exhibited spontaneous diabetes. If these surprising studies prove correct and are substantiated by other workers, they would strongly suggest that environmental factors modify genetic patterns involved in the production of diabetic states.

Long, Earl E.; and James, George (Akron City Health Dept., Akron, Ohio; Deputy Commissioner of Health, New York, N. Y.): COMMUNITY DETECTION OF DIABETES. Ohio M. J. 53:1280-83, November 1957.

Community diabetes detection programs do reveal a significant number of previously unknown diabetics, the authors conclude. The yield can be increased up to ten times when all those screening positive are followed up with at least a three-hour glucose tolerance test. The screening procedure can be adjusted to detect or miss the "potential diabetic" group with little or no effect on the number of previously unknown diabetics found.

Loustau-Chartez, Marc; and Vasseur, Bernard (Paris, France): HYPOGLYCEMIC SULFAMIDES AS TREATMENT FOR DIABETIC TUBERCULOUS PATIENTS. Presse méd. 66:1022-23, June 7, 1958.

The authors undertook treatment of diabetic tuberculous patients with carbutamide (BZ-55). They observed remarkable tolerance of hypoglycemic sulfamides. This allows for digressions both in diet and regimen that would have been hazardous under insulin treatment. (French)

Luft, R.; Ikkos, D.; Gemzell, C. A.; and Olivecrona, H. (Dept. Endocrinol., Serafimerlasarettet; Dept. Obst. & Gynec., Karolinska sjukhuset; and Dept. Neurosurg., Serafimerlasarettet, Stockholm, Sweden): EFFECT OF HUMAN GROWTH HORMONE IN HYPOPHYSECTOMIZED DIABETIC SUBJECTS. Lancet *1*:721-22, April 5, 1958.

The authors report on the administration of growth hormone purified from human pituitaries to three hypophysectomized diabetic patients. In all three the hormone administration was followed by a pronounced increase in blood sugar level, glycosuria and acetonuria. In two of the patients the administration had to be stopped after thirty-six hours.

Macho, L. (Endocrinological Inst., Slovak Academy of Sciences, Bratislava, Czechoslovakia): THE INFLUENCE OF ENDOCRINE GLANDS ON CARBOHYDRATE METABOLISM. I. THE DEFINITION AND ESTIMATION OF THE CLEARANCE OF GLUCOSE. Acta med. scandinav. *160*:477-84, May 6, 1958.

Clearance of glucose is defined as a volume of extracellular fluid cleared in unit of time of glucose in all the sites of the organism where glucose is utilized. The clearance values are in good correlation with the assimilation coefficient K and are inversely proportional to the time at which glycemia declines to the fasting value and to the natural logarithm of the concentration of blood sugar at sixty minutes. The average values of assimilation coefficient K in healthy subjects are 1.69 ± 0.27, of CL total 220.5 ± 54.4 ml./min. CLkg. 3.29 ± 0.92 ml./min./kg. body weight.

Macho, L. (Endocrinological Inst., Slovak Academy of Sciences, Bratislava, Czechoslovakia): THE INFLUENCE OF ENDOCRINE GLANDS ON CARBOHYDRATE METABOLISM: II. THE GLUCOSE TOLERANCE AND CLEARANCE OF GLUCOSE IN HEALTHY SUBJECTS AND IN PATIENTS WITH HYPO- AND HYPERTHYROIDISM. Acta med. scandinav. *160*:485-90, May 6, 1958.

A rapid intravenous glucose tolerance test was used to determine the rate at which glucose was utilized. Utilization was evaluated by determining the assimilation coefficient and clearance of glucose. A lowered and retarded rate of utilization of glucose was found in patients after thyroidectomy and with untreated hypothyroidism. In patients with treated hypothyroidism the rate increased. In patients with uncomplicated and untreated hyperthyroidism the rate was in the normal range.

Mailman, Richard A. (Med. Serv., Wadsworth Hosp., Veterans Administration Center, Los Angeles, Calif.): STEATORRHEA WITH DIABETES: A CASE REPORT. Ann. Int. Med. *49*:190-92, July 1958.

A case of steatorrhea is reported in a fifty-seven-year-old male diabetic with severe, poorly controlled diabetes of thirty-five years' duration. Long-term treatment with prednisone resulted in remission of the diarrhea.

Martin, Donald B.; Renold, Albert E.; and Dagenais, Yves M. (Baker Clin. Res. Lab.; Harvard Med. Sch.; Peter Bent Brig-

ham Hosp., Boston, Mass.): AN ASSAY FOR INSULIN-LIKE ACTIVITY USING RAT ADIPOSE TISSUE. Lancet 2:76-77, July 12, 1958.

The authors believe it is probable that the epididymal fat pad of the male rat can be successfully used for the assay of insulin-like activity in biological fluids. This method, in the hands of the authors, has proved more sensitive, more convenient and perhaps more accurate than methods previously described. It is, however, only a measure of insulin-like activity, not of insulin per se, and more experience is needed for a fuller appreciation of its adaptability and limitations.

Mehnert, Hellmut; Camerini-Dávalos, Rafael; and Marble, Alexander (Joslin Clinic and New England Deaconess Hosp., Boston, Mass.): RESULTS OF LONG-TERM USE OF TOLBUTAMIDE (ORINASE) IN DIABETES MELLITUS. J.A.M.A. 167: 818-27, June 1958.

Tolbutamide (Orinase) was used in treatment of 1,030 diabetic patients. In 772 of these, treatment with tolbutamide was begun and, at the time of completion of the study, had been carried out for one to twenty months in 594 patients. Good control of hyperglycemia and glycosuria was obtained in 407 (52.6 per cent) and fair control in 143 (18.5 per cent) of this selected group of 772 patients. The incidence of failures was as follows: "primary," that is, within the first month of treatment, 136 patients (17.6 per cent); "secondary," thirty-one patients (4 per cent); failures due to disregard of diet, nine patients (1.2 per cent). In thirty-three patients (4.3 per cent) the drug had been used for less than one month; in eight (1.1 per cent) treatment with tolbutamide was stopped because of toxic side effects; and in five (0.6 per cent) the treatment was discontinued for nonmedical reasons.

In the selection of patients to be treated with tolbutamide, care should be taken to avoid unnecessary use in those with whom dietary restriction will suffice and unwise use in those requiring insulin to maintain control of diabetes. Factors favoring success with tolbutamide were primarily maturity-onset type of diabetes (onset usually above the age of forty years) and a low insulin requirement (less than 20 units daily). A single-dose, four-hour sulfonylurea response test was found to be of value in pointing out those patients in whom primary failure with tolbutamide therapy is likely to occur.

Untoward effects were low in incidence and in general consisted chiefly of urticaria and minor digestive disturbances. In one patient with cirrhosis of the liver, jaundice occurred three months after beginning treatment with tolbutamide; the relation of jaundice to the drug is uncertain. In two patients dying of causes unrelated to tolbutamide administration, histological examination revealed no evidence of damage to the pancreas or liver that could be attributed to the drug.

Meyer, John S.; and Portnoy, Harold D. (Wayne State University, Coll. of Med., and Detroit Receiving Hosp., Detroit, Mich., and Veterans Administration Hosp., Dearborn, Mich.): LOCALIZED CEREBRAL HYPOGLYCEMIA SIMULATING STROKE: A CLINICAL AND EXPERIMENTAL STUDY. Neurology 8:601-14, August 1958.

Clinical and experimental evidence is presented to show that in the presence of a relative area of ischemia of the brain, hypoglycemia produces a localized reversible paralysis of neuronal function, with resultant motor and sensory deficit. During this stage of paralysis, metabolic utilization of oxygen is impaired. Administration of glucose restores normal functional activity and oxygen consumption to the area of hypoglycemic brain. It is presumed that the localized neuronal paralysis is the result of a reduced availability of glucose in the ischemic area and that the administration of glucose rapidly restores the deficient substrate. If sufficiently prolonged, localized hypoglycemia becomes irreversible and necrosis of nerve cells occurs.

Mirsky, I. Arthur (Pittsburgh, Pa.): DRUGS EFFECTIVE ORALLY IN THE TREATMENT OF DIABETES MELLITUS. A.M.A. Arch. Indust. Health 17:392-98, May 1958.

Irrespective of how tolbutamide causes a decrease in the blood sugar concentration, it is effective in the treatment of patients with the maturity-onset type of diabetes mellitus. The data to date suggest that from 60 per cent to over 90 per cent of patients who develop diabetes after the age of forty respond to approximately 1 gm. of tolbutamide per day. Such facts suggest that even more effective chemotherapeutic agents may become available in the future.

Mirsky, I. Arthur (Dept. of Clinical Science, Univ. of Pittsburgh Sch. of Med., Pittsburgh, Pa.): ORALLY EFFECTIVE HYPOGLYCEMIC AGENTS. Pennsylvania M. J. 61:861-66, July 1958.

It is quite probable that there are a number of reasons for the hypoglycemic response to the sulfonylureas, the author says. The apparent discrepancy among conclusions reached from different approaches may be more apparent than real. Thus the difference between the peripheral effects of tolbutamide and of insulin in the intact animal may be related to (1) the way the insulin is administered; (2) a restriction of the action of endogenous insulin to the liver since a decrease in glucose output occurs after the administration of insulin to man and dog; or (3) an interference with some of the actions of insulin in the presence of tolbutamide. Prior to the introduction of the sulfonylureas, many other agents were shown to be effective in reducing the hyperglycemia and glycosuria of patients with diabetes. Unlike the sulfonylureas, most of these agents are effective in the absence of islet tissue. The general application of these agents to the treatment of diabetes mellitus was usually precluded by their evident toxicity. A notorious exception, however, was the guanidine derivative called Synthalin. In spite of the noxious signs and symptoms which it produced in many patients, e.g., nausea, vomiting and a variety of other gastrointestinal disturbances, Synthalin was used quite extensively in the treatment of patients with diabetes until it became evident that it produced severe hepatic and renal damage.

In spite of the voluminous literature on the toxicity of the guanidine derivatives and in spite of the similarity between the noxious symptoms produced by Synthalin and those following the administration of the new guanidine derivative, many hundreds of patients with diabetes are being treated with the diguanide. The favorable reports on the use of phenethyldiguanide are reminiscent of the similar reports on the efficacy of Synthalin in the treatment of the patient with diabetes. As with the guanidine derivatives, interest has been revived recently in the use of acetylsalicylic acid in the treatment of diabetes. This hypoglycemic action of salicylates was extensively studied in the past. They were discarded as a therapeutic agent not only because of the toxicity they induced but also because of the way they produced their effects.

It would be superfluous to discuss the many plant and bacterial extracts that have been reported to be effective in producing a hypoglycemic response in animal and man. Of special interest, however, is the relatively new compound, Hypoglycin A. This very potent hypoglycemic agent is extremely toxic and is believed by some to be responsible for the Jamaican disease "vomiting sickness." Like many other toxic hypoglycemic agents, this amino acid is effective in the absence of functioning islets of Langerhans and irreversibly inactivates many enzyme systems. Its application to man can be awaited with much trepidation. Since insulin appears to act by enhancing the action of a transport system in the wall of the cell, it is conceivable that agents which can affect the transport system specifically should also be effective in the treatment of diabetes mellitus. Such agents are still unknown.

Moretti, G.; Geyer, A.; and Staeffen, J. (Bordeaux, France): RESEARCH STUDIES ON THE CELL CONTENTS OF LANGERHANS ISLETS IN DIABETES MELLITUS. Presse méd. 65:987-89, May 25, 1957.

The authors demonstrated in several cases of diabetes mellitus a fairly significant increase in the proportion of A cells —26 per cent—on an average against 15 per cent found in a control group of subjects with normal glycemia level. After a detailed description of the technic, eight illustrative clinical cases are summarized.

Moss, James M. (Georgetown Univ., Washington, D. C.): THE CLINICAL USE OF DEXTROTEST. Virginia M. Month. 85: 255-57, May 1958.

A rapid and economical blood sugar test is described. Dextrotest can be used in the detection, diagnosis and regulation of diabetes mellitus. It is especially helpful in emergency situations such as hypoglycemia or diabetic acidosis.

Nath, M. C.; and Khanade, J. M. (University Dept. of Biochemistry, Nagpur, India): LIVER KETOGENESIS: ROLE OF GLUCOSE-CYCLO-ACETOACETATE ON KETOGENESIS FROM FATTY ACIDS AND PYRUVATE. Proc. Soc. Exper. Biol. & Med. 97:706-09, March 1958.

The authors have demonstrated in the past that a condensation product of acetoacetate with glucose—glucose-cyclo-acetoacetate—acts to prevent the development of various types of experimental diabetes induced by alloxan, dehydroascorbic acid and acetoacetate. In this article the authors demonstrate that glucose-cyclo-acetoacetate prevents ketogenesis by the liver, increases the utilization of pyruvate by the liver and diaphragm, and apparently has no effect on the dissimilation of acetoacetate and β-hydroxybutyrate.

Newcomb, Alvah L.; Traisman, Howard S. (Dept. of Pediatrics, Northwestern Univ. Med. School, Children's Memorial Hosp., Chicago, Ill.): EFFECT OF CARBUTAMIDE IN JUVENILE DIABETES MELLITUS. Quart. Bull. Northwestern Univ. M. School 31:33-36, Spring 1957.

A report of the clinical trial of carbutamide in five juvenile diabetics in whom insulin therapy was continued. The authors found the drug of little value in the management of the child with diabetes mellitus.

Nichols, Nancy (Dept. of Medicine, Harvard Med. Sch., and Baker Clin. Res. Lab., New England Deaconess Hosp., Boston, Mass.): INTRACELLULAR GLYCOGEN AND ELECTROLYTE CONCENTRATIONS IN HUMAN SKELETAL MUSCLE. Proc. Soc. Exper. Biol. & Med. 97:363-66, February 1958.

The author describes the intracellular concentrations of sodium, potassium and phosphate glycogen found in six human subjects. The tissue was removed from the rectus abdominus muscle at surgery, and although the patients are considered normal their diagnoses varied from carcinoma of the sigmoid to pericolic abscess. The author's data are consistent with those previously reported in literature. One of the major differences noted was the glycogen content of human skeletal muscle. As determined by Mosketti the range was from 0.4 to 0.6 gm. per cent whereas the author found levels of glycogen ranging from 0.5 gm. per cent to 1.6 gm. per cent.

Paul, Jerome T. (Clinical Associate Professor of Medicine, University of Illinois Coll. of Medicine; Attending Physician, St. Francis Hosp., Evanston, Ill.): FRAGILE DIABETES. M. Clin. North America 25-30, January 1958.

The fragile type of diabetes is usually seen in the young or juvenile diabetic but is occasionally seen in the adult. Management is possible with careful selection of diet, choice of insulin and times of injections, and by understanding of the problems of the patient and education of the patient and the family.

Paul, Jerome T.; and Presley, Sophie J. (Dept. Med., Univ. of Illinois, Coll. of Med., and Res. and Educational Hospitals, Chicago, Ill.): COMPLICATIONS OF LONG-TERM DIABETES MELLITUS. Ann. Int. Med. 49:142-50, July 1958.

An analysis was made of 137 diabetics of long duration. Onset was below age fifty in 80 per cent. The incidence of small vessel complications (eye and kidney) and hypertension showed a significant increase with poor control as compared with good control. The age of onset and duration of the diabetes were also contributing factors.

Rapela, C. E.; and Gordon, Maria F. (Instituto de Biologia y Medicina Experimental, Costa Rica, Buenos Aires, Argentina): EFFECT OF INSULIN ON THE ADRENAL GLAND OF THE TOAD. Acta physiol. latinoam. 7:58-67, 1957.

The effect of insulin (20 U/100 gm. body weight) on blood sugar, weight, histology and catechol content of the adrenal glands of the toad, *Bufo arenarum Hensel,* was studied. The following changes were observed:

1. An initial fall of blood sugar occurred one to five hours after injection, followed by partial recovery. A second fall of glycemia was observed between twenty-four and forty-eight hours. About seventy-two hours after insulin injection, the blood sugar started to increase slowly, reaching normal levels on the fifth day. Convulsions were observed between twenty-four to forty-eight hours, but not during the first hypoglycemia (one to five hours).

2. The weight of the adrenals increased between the seventh and twentieth hour and was diminished forty-eight hours after the injection of insulin. Between the third and seventh day the adrenal glands of treated toads were heavier than the controls.

3. Size and vacuolization (lipoids) of the corticoadrenal cells were increased between ten and twenty-four hours, when the glands were heavier. Forty-eight hours after insulin administration, decrease of size of cortical cells and absence of vacuolization were observed.

4. Hypophysectomized toads injected with insulin showed

qualitatively similar but less intense histological changes of the adrenocortical cells than intact insulin treated animals.

5. No significant changes of the epinephrine and norepinephrine content of the adrenal glands were observed after insulin administration. These findings are opposite to those observed in mammals and birds.

Robinson, R. M.; Harris, Keith; Hlad, C. J.; and Eiseman, B. (Depts. of Surgery and Radioisotopes, Univ. of Colorado Med. Sch. and Denver Veterans Admin. Hosp., Denver, Colo.): EFFECT OF GLUCAGON ON GASTRIC SECRETION. Proc. Soc. Exper. Biol. & Med. 96:518-20, November 1957.

The administration of glucagon intravenously in a series of seventeen patients, of which five were controls, resulted in decreased gastric motility, gastric total secretion and hydrochloric acid production. This is presented as evidence against any etiologic relationship between glucagon and the ulcerogenic tumor of the pancreas as postulated by Zollinger and Ellison.

Rogers, Terence A. (Dept. of Physiol., University of Rochester Sch. of Medicine and Dentistry, Rochester, N. Y.): INHIBITION OF GLUCOSE UPTAKE BY ACIDOSIS IN VITRO. Proc. Soc. Exper. Biol. & Med. 97:647, March 1958.

The author demonstrates in the rat hemidiaphragm that when the insulin concentration in the medium exceeded a certain amount, the *p*H of the medium apparently did not influence the glucose uptake. But with decreasing insulin concentration in the medium, the muscles took up significantly less glucose at the lower *p*H's. Increasing the *p*H of the medium to 7.8 did not enhance glucose uptake by the diaphragm.

Salter, J. M.; De Meyer, R.; and Best, C. H. (The Banting and Best Dept. of Med. Res., Univ. of Toronto, Toronto, Ontario, Canada): EFFECT OF INSULIN AND GLUCAGON ON TUMOUR GROWTH. Brit. M. J. 2:5-7, July 5, 1958.

The authors describe the effect of insulin and glucagon on tumor growth (Walker carcinosarcoma tissue) in female Wistar rats. In a large series of other trials not reported here, insulin consistently produced a small but significant slowing of the tumor growth. This inhibition occurred only when the food intake of the treated animals was restricted to the level of the controls. The retardation of growth of the tumor induced by glucagon alone varied from 20 to 40 per cent. This inhibition can be attributed, in part, to the reduction in food intake and the adverse effect of glucagon on the growth of the rat as a whole. Although rats treated with both insulin and glucagon lose less weight than those given glucagon alone, inhibitions of tumor growth of 60 to 70 per cent obtained invariably. The inhibition is not therefore proportional to the weight loss. In each experiment the carcinostatic action of the combined therapy was much greater than the sum of the individual effects of the two substances. The authors suggest a clinical trial of the substances if the extensive inhibition of tumor growth by insulin and glucagon together is observed in a large number of other tumors in various species of animals.

Shallenberger, Paul L.; and Kapp, David F. (Dept. of Gastroenterology, Guthrie Clin., and Robert Packer Hosp., Sayre, Pa.): ACUTE PANCREATITIS: A CLINICAL REVIEW OF SEVENTY-TWO ATTACKS OCCURRING IN FIFTY-FOUR PATIENTS. Ann. Int. Med. 48:1185-93, June 1958.

In seventy-two attacks of acute pancreatitis among fifty-four patients, hyperglycemia was found in 53 per cent, with considerable fluctuation in the blood sugar values on repeated determination. Of forty-five survivors, 20 per cent subsequently developed diabetes. None of these had a family history of this disease.

Shreeve, Walton W. (Med. Dept., Brookhaven National Lab., Upton, N. Y., and the Radioisotope Unit, Crile Veterans Administration Hosp., Cleveland, Ohio): PATHWAYS OF CARBOHYDRATE FORMATION IN MAN. I. ISOTOPE DISTRIBUTION IN GLUCOSE FROM NONDIABETIC SUBJECTS GIVEN I-C^{14}-ACETATE. J. Clin. Invest. 37:999-1005, July 1958.

Carboxyl-labeled sodium acetate was used to assess the pathway of glucose metabolism in five diabetics. Of these, two were young labile diabetics in marked ketosis and three were stable, elderly diabetics. The carbohydrate metabolism of these patients utilized the same pathways as in the nondiabetics, namely, the Embden-Meyerhof and, to a lesser extent, pentose phosphate pathways.

Shreeve, Walton W.; and Hennes, Allen R. (Med. Dept., Brookhaven National Lab., Upton, N. Y.): PATHWAYS OF CARBOHYDRATE FORMATION IN MAN. II. THE EFFECT OF DIABETES AND GLUCOCORTICOID ADMINISTRATION ON ISOTOPE DISTRIBUTION IN GLUCOSE FROM SUBJECTS GIVEN I-C^{14}-ACETATE. J. Clin. Invest. 37:1006-15, July 1958.

Carboxyl-labeled sodium acetate was injected into four patients with carcinoma (lung or colon). The pathway of carbohydrate metabolism was inferred from the position of the labeled carbon in the blood glucose collected two hours later. Quantitatively the results are similar to those of other normal mammals: The main pathway is the classical Embden-Meyerhof with a low percentage by other (i.e., pentose phosphate) pathways.

Sobel, G. W.; Rodriguez-Inigo, J.; Morton, J. V.; and Levine, R. (Dept. of Metabolic & Endocrine Res., Med. Res. Inst., Michael Reese Hosp., Chicago, Ill.): STUDIES ON THE ACTION OF THE SULFONYLUREAS IN LIVERLESS DOGS. Metabolism 7:222-26, May 1958.

Hepatectomized dogs maintained on parenteral glucose, 150 mg./kg., exhibited a hypoglycemic response to tolbutamide similar in magnitude to that obtained in normal dogs. This lends further support to a pancreatotropic action of the drug.

Sorce, Richard C.; and Whitstone, William E. (Pittsburgh, Pa.): PRIMARY ALDOSTERONISM WITH DIABETES MELLITUS. A.M.A. Arch. Int. Med. 102:131-33, July 1958.

A case manifesting the features of primary aldosteronism and diabetes mellitus is presented. The diagnosis can be made from clinical findings and the demonstration of hypokalemic alkalosis and hypernatremia. Although aldosterone determinations are not yet available in most hospitals, selected patients with mild diabetes can be surveyed with simple tests. The relationship between diabetes and aldosteronism is briefly discussed.

Sullivan, John F. (Dept. of Neurol., Neurosurgery & Psychiatry, New England Center Hosp. & Dept. of Medicine, Tufts University Sch. of Medicine, Boston, Mass.): THE NEUROPATHIES OF DIABETES. Neurology 8:243-49, April 1958.

Diabetic neuropathy assumes one of two forms—(1) an asymmetric, painful, predominantly motor neuropathy, or (2)

a symmetric, distal, predominantly sensory neuropathy. It is acknowledged that patients with diabetes mellitus may have pain syndromes in various parts of the body without objective findings, and that other patients are without symptoms and demonstrate absent Achilles reflexes and diminished vibratory sensibility. It seems reasonable, however, that those with pain, but without objective findings, properly belong with the group of asymmetric neuropathies. The author suggests that damage to roots or peripheral nerves is less severe or occurs in trunk segments, and thus it may be impossible to demonstrate motor or sensory findings in these cases. The second group seems to be symmetric distal neuropathy, again being milder in degree. Whereas the former occurs in diabetes which is inadequately controlled or of long duration, the latter is often the first evidence of mild diabetes and tends toward spontaneous recovery. The contrasting clinical syndromes are described and the conclusion is drawn that such contrast suggests a different pathogenesis.

Tagnon, R.; Corvilain, J.; and Scohy, L. (Clin. Méd. Hôp. Saint-Pierre and Lab. de Médicine Expérimentale, Université Libre de Bruxelles, Brussels, Belgium): INFLUENCE OF FRUCTOSE ON THE EOSINOPHENIC REACTION FOLLOWING INSULIN ADMINISTRATION. Rev. franç. études clin. et biol. 3:615-17, June 1958.

The intravenous administration of fructose prevented the fall of blood eosinophils which follows the injection of insulin in fourteen normal subjects. The authors believe this fact demonstrates that the hypothalamus is able to utilize fructose.

Turner, Oliver E.: DIABETES—AN INCREASING PROBLEM. Allegheny County Medical Bulletin, June 8, 1957.

In 1950 diabetes was the twenty-third leading cause of death in the United States. In 1956, diabetes stood as the eighth leading cause of death, and 26,340 persons died from the disease. If the cardiovascular, renal and other complications were included, the figures would be much higher. Diabetes has increased considerably in the past thirty-five years, both in the new cases annually, and total number of cases. The cardiovascular, renal and eye complications of diabetes make the disease a real potential threat to the medical well-being of our future population, and therefore the medical profession should adjust its sights on the increasing need for early diagnosis of diabetes mellitus.

Tyberghein, Jean M.; and Williams, Robert H. (Dept. of Medicine, University of Washington Sch. of Medicine, Seattle, Wash.): METABOLIC EFFECTS OF PHENETHYLDIGUANIDE, A NEW HYPOGLYCEMIC COMPOUND. Proc. Soc. Exper. Biol. & Med. 96:29-32, October 1957.

The authors present some of the original metabolic observations reported on phenethyldiguanide (DBI). Guinea pigs were the experimental animals used for these observations. When DBI is administered to intact guinea pigs, it produces hypoglycemia associated with depletion of liver glycogen, but without increase in muscle glycogen. It also decreases the rate of formation of blood urea and decreases the nonprotein nitrogen excretion in the urine. It produces an early and definite rise in blood lactic acid. In vitro studies using guinea pig liver slices indicate that DBI increases lactic acid formation and glycogen depletion, but does not affect glucose output on the isolated rat diaphragm. There is an increased glucose uptake, increased lactic acid production, decreased glycogen formation and decreasing oxygen uptake. These changes suggest that this compound acts by increased anaerobic glycolysis, which is different from insulin and the sulfonylurea compounds.

Waddell, William R. (Dept. of Nutrition, Harvard University Sch. of Public Health, Boston, Mass.): EFFECT OF TOLBUTAMIDE ON SERUM CHOLESTEROL LEVELS IN HYPERCHOLESTEROLEMIC PATIENTS. Proc. Soc. Exper. Biol. & Med. 98: 280, June 1958.

Eight patients with idiopathic hypercholesterolemia with control serum cholesterol levels ranging between 300 and 500 mg. per liter were given tolbutamide in a maintenance dose of 1 gm. per day for a period of two months. Although there was a slight decrease in serum cholesterol values during the first week of administration, in no patient was a serum cholesterol lowered below control level during the second month of tolbutamide therapy. It should be noted that the patients were on a relatively constant fat intake throughout the trial.

Weber, George; and Cantero, Antonio (Res. Labs., Montreal Cancer Inst., Notre Dame Hosp., Montreal, Canada): EFFECT OF ORINASE ON HEPATIC ENZYMES INVOLVED IN GLUCOSE-6-PHOSPHATE UTILIZATION. Metabolism 7:333-37, July 1958.

The authors confirm the effect of Orinase on liver glucose-6-phosphate in rats. They demonstrated a lack of effect of the drug on phosphoglucomutase and glucose-6-phosphate dehydrogenase. Since the utilization of glucose-6-phosphate is influenced by the relative activities of the specific liver enzymes which channel it into four possible pathways, a metabolic imbalance results from the presence of Orinase. The in vitro effect, they conclude, is compatible with a decreased glucose release, decreased glycolysis and increased glycogen deposition.

Wells, Ronald; and Yam, Tan Bock (Diabetes Clin., Genl. Hosp., Singapore): A CLINICAL TRIAL OF SULPHONILYL-BUTYLCARBAMIDE IN FIFTY-ONE DIABETICS. Proc. Alumni A., Malaya 10:124-34, June 1957.

The results are described of a short trial of fifty-one selected diabetics on sulphonilyl-butylcarbamide (BZ-55). The most significant findings were that in the thirty-six patients previously stabilized on insulin, fifteen were better controlled on sulphonilyl-butylcarbamide and nineteen were controlled to the same extent on both drugs. The toxic and hypoglycemic effects noticed were uncommon and minimal. It must also be realized that these patients had only been tried on small initial doses of sulphonilyl-butylcarbamide for a short period, and in several cases larger doses might have been given even better control. It remains to be seen whether the long-term results of sulphonilyl-butylcarbamide therapy are as satisfactory as those obtained with insulin. But until convincing evidence is produced to the contrary it will become increasingly difficult to convince the doctors and patients who have tried the new drugs that they should go on using insulin.

ORGANIZATION SECTION

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Seventh Postgraduate Course in Diabetes and Basic Metabolic Problems

St. Louis, Missouri, Jan. 21-23, 1959

Preliminary Program

The American Diabetes Association will hold its Seventh Postgraduate Course in Diabetes and Basic Metabolic Problems in St. Louis, Missouri, Jan. 21, 22 and 23, 1959. Open to Doctors of Medicine, the Course was developed by the Committee on Professional Education of the American Diabetes Association, and will be given at the Chase-Park Plaza Hotels.

T. S. Danowski, M.D., Pittsburgh, is Chairman of the Program Committee; Frank N. Allan, M.D., Boston, and Robert H. Williams, M.D., Seattle, are Vice Chairmen. The Course will be given in co-operation with the St. Louis University School of Medicine, Washington University School of Medicine, and the University of Missouri School of Medicine.

William H. Olmsted, M.D., St. Louis, is Director of the Course with Henry E. Oppenheimer, M.D., St. Louis, as Co-Director. The Local Committee on Arrangements includes: Representing St. Louis University: *Robert E. Fox, M.D., Ralph A. Kinsella, Jr., M.D., Henry E. Oppenheimer, M.D., Daniel L. Sexton, M.D.;* representing Washington University: *William H. Daughaday, M.D., Alexis F. Hartmann, M.D., W. Stanley Hartroft, M.D., Ph.D., Harold K. Roberts, M.D.;* representing University of Missouri: *Thomas W. Burns, M.D., Robert L. Jackson, M.D.*

Round-table Conferences, a new feature of the Postgraduate Course series, will be held January 22 at the close of the afternoon session. The purpose is to provide registrants with an opportunity to ask designated discussion leaders questions that may be concerning them. Groups will consist of no more than sixteen registrants. Discussion will be informal and may pertain to papers presented or any area of specific interest to the registrants or the leaders.

A Social Hour and a Banquet are scheduled for Wednesday evening, January 21, at the Chase-Park Plaza Hotels. Registrants and faculty members are guests of the American Diabetes Association at the Banquet and the Social Hour is by individual subscription. Another Social Hour will be held on Thursday evening, January 22, at 6:00 p.m.

REGISTRATION: The preliminary program that is being mailed to members of the Association and subscribers of the Journal includes an application form. This form should be filled out and mailed to the American Diabetes Association, Inc., 1 East 45th St., New York 17, N.Y. Applications will be accepted in the order received and registrations officially confirmed. Registration at the Course will be from 2:00 p.m. to 9:00 p.m., Tuesday, January 20, and will open again at 8:00 a.m., Wednesday, January 21. Everyone is urged to register before 9:00 a.m. on Wednesday in order that the Course may begin promptly.

FEES: The registration fee for the three-day Course is $50 for members of the American Diabetes Association, and $100 for nonmembers. The full fee must be paid when the Course application is filed but will be returnable by the Association to any registrant who submits his withdrawal in writing not later than January 9.

Fellows and residents engaged in full-time study of medicine and allied sciences in schools and hospitals in the St. Louis metropolitan area only *may attend the scientific sessions at the nominal fee of $10; interns may attend without charge. Fellows, residents and interns should register by January 9. Medical students in the metropolitan area may register without charge after January 9 if space permits.*

HOTEL ACCOMMODATIONS: The Chase-Park Plaza Hotels will serve as headquarters. Reservation cards will be sent to registrants with their matriculation card confirming acceptance for the Course.

POSTGRADUATE CREDIT: The American Academy of General Practice will give eighteen hours' Postgraduate Credit for the Course (Category II).

THE FOLLOWING TOPICS AND DISCUSSIONS (SUBJECT TO CHANGE) WILL BE COVERED DURING THE 3-DAY COURSE.

WEDNESDAY MORNING, JANUARY 21

9:00 Introduction by Co-Directors—William H. Olmsted and Henry E. Oppenheimer
Welcome—Alexander Marble, President

THE PATHOPHYSIOLOGY OF INSULIN: Chairman, Francis D. W. Lukens

9:10 The Physiology of Insulin—William C. Stadie
9:40 The Release of Insulin—Arnold Lazarow
10:00 *Intermission*
10:20 The Hormonal Factors Influencing Insulin Action—Francis D. W. Lukens
10:50 Nonhormonal Antagonists of Insulin—Hiromichi T. Narahara
11:10 to 12:00 *Panel*: Factors Influencing Insulin Action—Francis D W. Lukens, Moderator; Arnold Lazarow, Hiromichi T. Narahara, W. Stanley Hartroft, William H. Daughaday

WEDNESDAY AFTERNOON, JANUARY 21

THE TREATMENT OF DIABETES: GENERAL PRINCIPLES: Chairman, Frank N. Allan

1:30 The Correlation of Islet Pathology with Clinical Diabetes—W. Stanley Hartroft
2:00 The Use of Insulins and the Goals in the Control of Hyperglycemia—Arthur R. Colwell, Sr.
2:30 Spontaneous Hypoglycemia as an Early Manifestation of Diabetes—Jerome W. Conn
3:00 *Intermission*
3:20 The General Principles of Dietetic Management—Garfield G. Duncan
3:40 Special Dietetic Management of Children—Alexis F. Hartmann
3:55 to 5:00 *Panel*: The Technics of Dietetic Management—Garfield G. Duncan, Moderator; Jerome W. Conn, Alexander Marble, Frank N. Allan, William H. Olmsted

6:30 Social Hour
7:30 Banquet

THURSDAY MORNING, JANUARY 22

THE RELATIONSHIP OF LIPID METABOLISM TO DIABETES: Chairman, Thomas P. Sharkey

9:00 Obesity and Diabetes—Thomas W. Burns
9:15 Disturbances in Fat Metabolism—David M. Kipnis
9:45 Role of Lipids in Atherosclerosis—Theodore B. Van Itallie
10:15 *Intermission*
10:35 Diabetic Acidosis—William H. Daughaday
11:05 to 12:00 *Panel*: Interrelationships of Diabetes and Atherosclerosis—T. S. Danowski, Moderator; Thomas W. Burns, David M. Kipnis, Theodore B. Van Itallie, William H. Daughaday

THURSDAY AFTERNOON, JANUARY 22

SPECIAL PROBLEMS IN THE MANAGEMENT OF DIABETES: Chairman, John A. Reed

1:30 Types of Diabetes and Standards of Control—Henry T. Ricketts
2:00 Juvenile Diabetes—Robert L. Jackson
2:30 The Treatment of the Pregnant Diabetic—Max Miller
3:00 *Intermission*
3:30 to 5:00 *Round-Table Conferences*

LEADERS

Frank N. Allan	Rachmiel Levine
Charles H. Best	Francis D. W. Lukens
Arthur R. Colwell, Sr.	Alexander Marble
Jerome W. Conn	Max Miller
T. S. Danowski	Franklin B. Peck, Sr.
Garfield G. Duncan	Henry T. Ricketts
Robert M. Kark	William C. Stadie
Harvey C. Knowles, Jr.	Frederick W. Williams

FRIDAY MORNING, JANUARY 23

COMPLICATIONS OF DIABETES: Chairman, Franklin B. Peck, Sr.

9:00 The Ocular Complications of Diabetes—Bernard Becker
9:30 The Renal Complications of Diabetes—Robert M. Kark
10:00 Surgery of Obstructive Atherosclerosis—Carl A. Moyer
10:30 *Intermission*
10:50 Surgery and Transplantation of Obstructed Arteries—C. Rollins Hanlon
11:20 to 12:20 *Panel*: Diabetes During Acute Illness—Henry T. Ricketts, Moderator; John A. Reed, Robert M. Kark, Carl A. Moyer, E. Paul Sheridan

FRIDAY AFTERNOON, JANUARY 23

THE ORAL HYPOGLYCEMIC AGENTS: Chairman, E. Paul Sheridan

2:00 The History, Development and Chemistry of the Oral Hypoglycemic Agents—Lillian Recant
2:20 Mode of Action of the Oral Hypoglycemic Agents—Rachmiel Levine
2:40 Long-term Experimental Effects of the Oral Hypoglycemic Agents—Charles H. Best
3:00 *Intermission*
3:20 Clinical Application of the Oral Hypoglycemic Agents—Alexander Marble
4:00 to 5:00 *Panel*: The Place of the Oral Hypoglycemic Agents in the Treatment of Diabetes—Arthur R. Colwell, Sr., Moderator; Lillian Recant, Rachmiel Levine, Charles H. Best, Alexander Marble

FACULTY OF THE SEVENTH POSTGRADUATE COURSE

FRANK N. ALLAN, M.D., *Executive Director, Medical Department, Lahey Clinic; Member, Medical Administrative Board, New England Deaconess Hospital, Boston, Massachusetts.*

BERNARD BECKER, M.D., *Professor of Ophthalmology and Head of the Department, Washington University School of Medicine, St. Louis, Missouri.*

CHARLES H. BEST, C.B.E., M.D., F.R.S., *Professor of Physiology; Director and Professor in the Banting and Best Department of Medical Research, University of Toronto, Toronto, Ontario, Canada.*

THOMAS W. BURNS, M.D., *Associate Professor of Medicine, University of Missouri School of Medicine, Columbia, Missouri.*

ARTHUR R. COLWELL, SR., M.D., *Professor of Medicine and Chairman, Department of Medicine, Northwestern University Medical School, Chicago, Illinois.*

JEROME W. CONN, M.D., *Professor of Medicine and Director, Metabolism and Research Unit, University of Michigan Medical School; Chief, Department of Endocrinology and Metabolism, University Hospital, Ann Arbor, Michigan.*

T. S. DANOWSKI, M.D., *Professor of Research Medicine, The University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania.*

WILLIAM H. DAUGHADAY, M.D., *Associate Professor and Director of Metabolism Division, Department of Medicine, Washington University School of Medicine; Barnes Hospital, St. Louis, Missouri.*

GARFIELD G. DUNCAN, M.D., *Clinical Professor of Medicine, Jefferson Medical College; Director of the Medical Divisions, Pennsylvania Hospital and Benjamin Franklin Clinic, Philadelphia, Pennsylvania.*

C. ROLLINS HANLON, M.D., *Professor of Surgery and Director, Department of Surgery, St. Louis University School of Medicine; Surgeon in Chief, St. Louis University Hospitals, St. Louis, Missouri.*

ALEXIS F. HARTMANN, M.D., *Professor of Pediatrics and Head of the Department, Washington University School of Medicine; Physician in Chief, St. Louis Children's Hospital, St. Louis, Missouri.*

W. STANLEY HARTROFT, M.D., Ph.D., *Mallinckrodt Professor and Chairman, Department of Pathology, Washington University School of Medicine; Pathologist in Chief, Barnes and Allied Hospitals and St. Louis Children's Hospital, St. Louis, Missouri.*

ROBERT L. JACKSON, M.D., *Professor and Chairman, Department of Pediatrics, University of Missouri School of Medicine; Pediatrician in Chief, University Hospitals, Columbia, Missouri.*

ROBERT M. KARK, M.D., *Professor of Medicine, University of Illinois College of Medicine, Chicago, Illinois.*

DAVID M. KIPNIS, M.D., *Associate Professor and Markle Scholar in Biochemistry, Department of Medicine, Washington University School of Medicine, St. Louis, Missouri.*

HARVEY C. KNOWLES, JR., M.D., *Associate Professor of Medicine, University of Cincinnati College of Medicine, Cincinnati, Ohio.*

ARNOLD LAZAROW, M.D., *Professor and Head, Department of Anatomy, University of Minnesota Medical School, Minneapolis, Minnesota.*

RACHMIEL LEVINE, M.D., *Professorial Lecturer in Physiology, University of Chicago; Chairman, Department of Medicine and Director, Metabolic and Endocrine Research, Michael Reese Hospital, Chicago, Illinois.*

FRANCIS D. W. LUKENS, M.D., *Professor of Medicine and Director, George S. Cox Medical Research Institute, University of Pennsylvania, Philadelphia, Pennsylvania.*

ALEXANDER MARBLE, M.D., *Assistant Clinical Professor of Medicine, Harvard Medical School; Joslin Clinic and New England Deaconess Hospital, Boston, Massachusetts.*

MAX MILLER, M.D., *Associate Professor of Medicine, Western Reserve University School of Medicine, Cleveland, Ohio.*

CARL A. MOYER, M.D., *Bixby Professor of Surgery, Washington University School of Medicine, St. Louis, Missouri.*

HIROMICHI T. NARAHARA, M.D., *Research Fellow, Department of Biological Chemistry, Washington University School of Medicine, St. Louis, Missouri.*

WILLIAM H. OLMSTED, M.D., *Associate Professor of Clinical Medicine (Emeritus), Washington University School of Medicine; Barnes Hospital, St. Louis, Missouri.*

FRANKLIN B. PECK, SR., M.D., *Associate Professor of Medicine, Indiana University School of Medicine; Consultant in Medicine, Indianapolis General Hospital, Indianapolis, Indiana.*

LILLIAN RECANT, M.D., *Associate Professor of Medicine and Preventive Medicine, Washington University School of Medicine; Assistant Physician, Barnes and Allied Hospitals, St. Louis, Missouri.*

JOHN A. REED, M.D., *Assistant Clinical Professor of Medicine, George Washington University School of Medicine; Attending Physician, George Washington University Hospital, Washington, D.C.*

HENRY T. RICKETTS, M.D., *Professor of Medicine, University of Chicago; Attending Physician, Albert Merritt Billings Hospital, Chicago, Illinois.*

THOMAS P. SHARKEY, M.D., *Assistant Clinical Professor of Medicine, The Ohio State University College of Medicine; Consultant in Internal Medicine and Pathology, Miami Valley Hospital, Dayton, Ohio.*

E. PAUL SHERIDAN, M.D., *Assistant Clinical Professor of Medicine, University of Colorado Medical Center; St. Luke's Hospital, Denver, Colorado.*

WILLIAM C. STADIE, M.D., *Professor of Research Medicine, The John Herr Musser Department of Research Medicine, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania.*

THEODORE B. VAN ITALLIE, M.D., *Associate Clinical Professor of Medicine, Columbia University College of Physicians and Surgeons; Director of Medicine and Attending Physician, St. Luke's Hospital, New York, New York.*

FREDERICK W. WILLIAMS, M.D., *Associate Clinical Professor of Medicine, New York Medical College; Morrisania City Hospital, New York, New York.*

NINETEENTH ANNUAL MEETING

As previously announced, the Nineteenth Annual Meeting of the American Diabetes Association will be held in Atlantic City, New Jersey, June 6-7, 1959, prior to the annual session of the American Medical Association. An announcement of the Annual Meeting together with a hotel reservation card is being mailed to members of the Association.

SCIENTIFIC PROGRAM

Physicians and other scientists, who would like to present papers at the Scientific Sessions, are invited to submit abstracts to Franklin B. Peck, Sr., M.D., Chairman of the Committee on Scientific Programs. Eleven copies of the abstracts, which are requested in order to expedite review by the Committee, should be sent as soon as possible to Dr. Peck in care of the national office.

BOARD OF STATE GOVERNORS

New officers were elected to the Board of State Governors on June 21, 1958, at the time of the Eighteenth Annual Meeting Christopher J. McLoughlin, M.D., Atlanta, Georgia, was elected Chairman, to succeed Lewis B. Flinn, M.D., Wilmington, Delaware. By reason of this office, Dr. McLoughlin will serve as an ex officio member of the Council of the American Diabetes Association.

Morris Margolin, M.D., Omaha, Nebraska, was re-elected Vice Chairman, and Robert L. Reeves, M.D., Seattle, Washington, was elected Secretary of the Board.

Governors who will serve for the organizational year 1958-59 are:

Term Expiring June 1960

State	*Governor*
Alabama	Samuel Eichold, M.D.
Arizona	Eleanor Waskow, M.D.
Arkansas	Hal Dildy, M.D.
California	
Northern & Nevada	Leo Goodman, M.D.
Southern	Roy F. Perkins, M.D.
Colorado	W. Bernard Yegge, M.D.
Connecticut	Barnett Greenhouse, M.D.
Delaware	Lewis B. Flinn, M.D.
District of Columbia	Maurice Protas, M.D.
Florida	Sidney Davidson, M.D.
Georgia	Christopher J. McLoughlin, M.D.
Idaho	Glenn Q. Voyles, M.D.
Illinois	
Northern	Ford K. Hick, M.D.
Southern	Thomas D. Masters, M.D.
Indiana	Marion R. Shafer, M.D.
Iowa	Robert C. Hardin, M.D.

Term Expiring June 1961

Kansas	Eldon S. Miller, M.D.
Kentucky	Carlisle Morse, M.D.
Louisiana	Daniel W. Hayes, M.D.
Maine	Elton R. Blaisdell, M.D.
Maryland	Abraham A. Silver, M.D.
Massachusetts	C. Cabell Bailey, M.D.
Michigan	Laurence F. Segar, M.D.
Minnesota	Moses Barron, M.D.
Missouri	Henry E. Oppenheimer, M.D.
Montana	F. Hughes Crago, M.D.
Nebraska	Morris Margolin, M.D.
Nevada	See Northern California
New Hampshire	See Vermont
New Jersey	Benjamin Saslow, M.D.
New York	
Eastern	Alfred E. Fischer, M.D.
Western	Joseph L. Izzo, M.D.
North Carolina	William M. Nicholson, M.D.
North Dakota	Edgar A. Haunz, M.D.

Term Expiring June 1959

Ohio	Joseph I. Goodman, M.D.
Oklahoma	Bert F. Keltz, M.D.
Oregon	Wm. Richey Miller, M.D.
Pennsylvania	
Eastern	William F. Hanisek, M.D.
Western	John A. O'Donnell, M.D.
Rhode Island	Louis I. Kramer, M.D.
South Carolina	Robert Wilson, M.D.
South Dakota	Everett W. Sanderson, M.D.
Tennessee	Albert S. Easley, M.D.
Texas	George M. Jones, M.D.
Utah	Donald E. Smith, M.D.
Vermont &	
New Hampshire	Ellsworth L. Amidon, M.D
Virginia	William R. Jordan, M.D.
Washington	Robert L. Reeves, M.D.
West Virginia	Richard N. O'Dell, M.D
Wisconsin	Elwood W. Mason, M.D

ASSEMBLY OF DELEGATES

Henry E. Oppenheimer, M.D., of St. Louis, Chairman, and Mrs. Earl R. Hoover of Cleveland, Secretary, were re-elected to serve in these posts at the Annual Meeting of the Assembly of Delegates held on June 21, 1958, in San Francisco. By reason of this office, Dr. Oppenheimer serves as an ex officio member of the Council of the American Diabetes Association, and he is also the Association's Governor for the State of Missouri. Mrs. Hoover is a founder and Vice President of the Diabetes Association of Greater Cleveland.

1958-59 MEDICAL STUDENT-INTERN ESSAY CONTEST

The seventh Medical Student-Intern Essay Contest (1958-59) is open to medical students, interns and physicians within two years after their graduation from medical school.

The author or authors of the best paper reporting original work, whether laboratory investigation or clinical observation, will receive an award of $250. This prize again has been made possible by the St. Louis Diabetes Association.

One hundred dollars ($100), double the award in previous years, will be given for the best review article or case report.

The papers will be judged on the basis of the value of the material and the method of presentation. Any subject relating to diabetes and basic metabolic problems may be selected. Those entering should submit the original and two copies of their manuscripts. Manuscripts should be typewritten, double-spaced, and mailed by April 1, 1959, to: Committee on Scientific Awards, American Diabetes Association, Inc., 1 East 45th St., New York 17, N.Y.

Members of the American Diabetes Association and subscribers to DIABETES are asked to encourage medical students, interns and physicians within two years after their graduation from medical school to enter the contest.

JANUARY 1 DEADLINE FOR 1959 LILLY AWARD

Nominations for the third Lilly Award, which will be given at the Nineteenth Annual Meeting of the American Diabetes Association, June 6-7, 1959, in Atlantic City, New Jersey, must be received by the Association before Jan. 1, 1959. The following stipulations govern the contest for the annual award, which is supported by Eli Lilly and Company and consists of $1,000 and a medal.

Purpose: To recognize demonstrated research in the field of diabetes, taking into consideration independence of thought and originality.

Eligibility: Any investigator in an appropriate field of work closely related to diabetes who is less than forty years of age on January 1 of the year in which the award is made. The research will not necessarily be judged in comparison to the work of more mature and experienced workers. The candidate should be a resident of the United States or Canada.

Nominations: Nominations for the award will be solicited from the members of the American Diabetes Association. Such nominations will be requested by repeated notices to be published in DIABETES. Names of nominees will be sent to the Chairman of the Committee on Scientific Awards and must be received before January 1 of the year of the award. The nomination should be accompanied by full information concerning the nominee's personality, training and research work. Six copies of each item should be submitted. No member may send in more than one nomination. A list of the nominee's publications, if any, and six copies of the publication or manuscript for which the award is to be given should also accompany the nomination. At the discretion of the Committee on Scientific Awards, the award may be given for work published during the year prior to January 1 of the same year of the award. The nominee should be actively engaged at that time in the line of research for which the award is to be made.

Announcement: The name of the winner will be announced in the program of the Annual Meeting of the Association, and the award presented at that meeting. The winner, subject to the approval of the Committee on Scientific Programs, will be invited to present a paper on the subject of his work. Papers considered for the award must be submitted with the idea that they will be published in whole or in part in DIABETES if found acceptable to the Editor and/or Editorial Board. If the Committee should decide that no outstanding work has been presented for this consideration, the award will not be made.

Award: In addition to the monetary award and the medal, traveling expenses will be given to make it possible for the recipient to receive his award in person at the Annual Meeting.

NEW *ADA FORECAST* REPRINT SERIES

The Editors of FORECAST and the Committee on Information for Diabetics of the American Diabetes Association have reprinted ten additional articles from recent issues. They will help your patients and their families to understand the condition and thus to control it better. The new reprints are entitled: "The Oral Hypoglycemic Compounds"; "The Truth about Life Insurance for Diabetics"; "Does Sugar in the Urine Always Mean Diabetes?"; "Certification of Insulin"; "What Do You Want to Know about Diabetes?"; "The Seven Wonders of a Slice of Bread"; "Storm Warning!"; "A Behind-the-Scenes Look at Modern Insulin Production"; "Medical Quacks and Fakers"; "Diabetes and Arteriosclerosis."

Thirty-seven earlier reprints are also available. Write to American Diabetes Association, Inc., 1 East 45th St., New York 17, N.Y., for a complete list and an order form. All reprints are priced at ten cents each or three for twenty-five cents, which includes postage and handling. Order forms and quantity prices are available.

ADDENDUM ON TOLBUTAMIDE FOR *DIABETES GUIDE BOOK*

A statement on the uses of tolbutamide has been prepared as an addendum to the Second Edition of DIABETES GUIDE BOOK FOR THE PHYSICIAN. You may obtain a copy of the addendum without charge upon request.

New copies of the GUIDE BOOK which include the addendum are also available, and may be purchased singly or in quantity. The following prices have been established: 1 to 9 copies, $1.00 each; 10 to 24 copies, $.95 each; 25 to 49 copies, $.90 each; 50 to 99 copies, $.85 each; 100 to 249 copies, $.80 each; 250 or more copies, $.75 each.

Address your orders to the American Diabetes Association, Inc., 1 East 45th St., New York 17, N.Y. All prices include handling and shipping.

TALKING BOOK RECORDS FOR THE BLIND

The American Foundation for the Blind in conjunction with the Committee on Information for Diabetics of the American Diabetes Association has released new Talking Book transcriptions of nineteen outstanding articles from ADA FORECAST and three items of special interest to diabetics.

Titles of the FORECAST articles are: "Wonder Drugs and the Diabetic"; "Your Teeth"; "Diabetic Neuritis"; "Overcoming a Common Ailment"; "The Diabetic Foot"; "Healthy Babies for Diabetic Mothers"; "The Child with Diabetes"; "Types of Insulin" (incorporating the article "Lente: The New Insulin"); "The Care and Handling of Insulin Syringes"; "Watch Those Injection Sites"; "Insulin Reaction"; "Obesity" (including "All About Calories"); "The Food We Eat"; "The Six Exchange Lists" (with recipes from "The High Cost of Proteins"); "Sugar Substitutes"; "Canning Fruits without Sugar"; "Traveling with Diabetes" (incorporating "Meals on Wheels"); "Diabetics and the National Emergency"; "Beware of Fake Diabetes Cures."

Another article of special interest is entitled "The American Diabetes Association." Other items are "An Oral Treatment for Diabetes" and "The Blind Diabetic."

Like other Talking Books, copies of the set may be borrowed through the thirty regional libraries of the U.S. Library of Congress.

Individuals who wish to purchase their own set may do so by writing the American Foundation for the Blind, 15 West 16th St., New York 11, N.Y. The price for the four-record set is $4, postpaid.

ADA LISTED IN *GIVER'S GUIDE*

The American Diabetes Association is among fifty-nine health and welfare organizations listed in the 1958-59 *Giver's Guide to National Philanthropies* issued by the National Information Bureau. The Bureau, a nonprofit organization, was founded in 1918 "To maintain sound standards in national philanthropy" and "To advise and protect contributors."

The Bureau has available reports of nearly 600 nonprofit organizations and publishes the *Guide* to illustrate the types of accredited agencies which meet the standards of the Bureau.

Individual copies, as well as copies in quantity, may be secured from the National Information Bureau, 205 East 42nd St., New York 16, N.Y.

A VALUABLE LIBRARY

The *Proceedings of the American Diabetes Association,* containing a great body of clinical information of practical use to every physician treating diabetes, may be purchased at a special price of $34.50 for Volumes 2, 3, 4, 6, 7, 8, 9 and 10. Volumes 1 and 5 are out of print. For those who wish to complete their series, separate volumes may be obtained at $5.00 a copy. Please direct your orders to the American Diabetes Association, Inc., 1 East 45th St., New York 17, N.Y.

The *Proceedings,* previously published annually, was discontinued in 1950 with Volume 10. The publication was superseded by DIABETES, *The Journal of the American Diabetes Association.*

ADA FORECAST RECIPE BOOK

Your diabetic patients, their families and friends will be gratified to know that a book of recipes and menus by Deaconess Maude Behrman will soon be published by the American Diabetes Association. Indexed and styled for ready use, it contains hundreds of food specialties expressly written for diabetics. Additional information including the date of publication will be announced.

NEW MEMBERS

Active

The following were elected as of Oct. 1, 1958, and Nov. 1, 1958:

Alabama
Sarrell, Warren G. — Anniston

Colorado
Mullett, Aidan M. — Colorado Springs

District of Columbia
Canary, John J. — Washington

Georgia
Hirsch, Jack W. — Columbus

Illinois
Metz, Robert J. S. — Chicago
Skom, Joseph H. — Chicago

Kansas
Stoffer, Robert P. — Halstead

Massachusetts
Massarelli, John J., Jr. — Worcester
Rengarts, Robert T. — Boston

Nebraska
Seng, Willard G. — Oshkosh

New York
Carlozzi, Michael — Brooklyn
Iezzoni, Domenic G. — Brooklyn
Jansons, Voldemars — Ridgewood
Rachow, Donald O. — Snyder
Sturman, Martin F. — New York
Ulrich, Herbert J. — Buffalo

Ohio
Baumgardner, Howard T. — Akron

Oklahoma
Adams, George — Oklahoma City

Pennsylvania
Masland, David S. — Carlisle
Schmidt, William A. — Shenandoah
Simkins, Samuel — Philadelphia

South Carolina
Buse, John F., Jr. — Charleston

Tennessee
Liddle, Grant W. — Nashville

Virginia
Tyroler, Sidney A. — Arlington

Other Countries

Argentina
Sarsotti, Carlos I. — Santa Fe

Italy
Devide, Antonio — Treviso

Switzerland
Curchod, Bernard P. — Lausanne
Schertenleib, Fred E. — Bern

NEWS OF AFFILIATE ASSOCIATIONS

The GEORGIA DIABETES ASSOCIATION presented a one-day postgraduate program on diabetes for general practitioners in Atlanta on Oct. 17, 1958, immediately following the 10th Annual Session of the Georgia Academy of General Practice. The morning program was moderated by Alex T. Murphey, M.D., and the following papers were given: "Diagnosis of Diabetes," by Frederick B. Cheney, M.D.; "Bedside Blood Sugar Determinations," by Harold A. Ferris, M.D.; "Childhood Diabetes," by C. W. Cary Moore, M.D.; "Oral Hypoglycemic Agents," by Guy J. Dillard, M.D.; "Selecting the Proper Insulin," by Wm. L. Paullin, Jr., M.D.; "Dietary Control of Diabetes," by Jules Victor, Jr., M.D.; "Insuring the Diabetic Patient," by Mr. J. E. K. Kennedy.

Edwin C. Evans, M.D., moderated the afternoon program which included: "The Pregnant Diabetic Patient," by C. J. McLoughlin, M.D.; "Diabetes Acidosis," by Harvey C. Knowles, Jr., M.D., Cincinnati; "Differential Diagnosis of Renal Disease in Diabetes," by Joseph Wilson, M.D.; "Peripheral Vascular Disorders in Diabetes," by John K. Davidson, M.D.; panel discussion moderated by C. Raymond Arp, M.D., entitled "Complications and Stress in Diabetes," with Harvey C. Knowles, Jr., M.D., Walter L. Bloom, M.D., John K. Davidson, M.D., A. Park McGinty, M.D., Thomas Findley, M.D., and Alex T. Murphey, M.D.

The INDIANAPOLIS DIABETES ASSOCIATION (Clinical Society) held a dinner meeting November 13, at which Alexander Marble, M.D., Boston, spoke on "The Management of Juvenile Diabetes in Relation to Late Sequelae."

The MICHIGAN DIABETES ASSOCIATION (Clinical Society) presented a scientific program October 3 in Detroit. Presiding at the afternoon session was William L. Lowrie, M.D., Chairman of the Clinical Society, and at the evening session Frank S. Perkin, M.D., President, Michigan Diabetes Association.

The afternoon program included: "Oral Hypoglycemic Agents in the Management of Diabetes," by Fred W. Whitehouse, M.D., Associate Physician, Henry Ford Hospital, Detroit; "Reasons for Caution in the Use of Tolbutamide," by Keats K. Vining, Jr., M.D., Attending Physician, Blodgett Hospital, Grand Rapids; "Management of Diabetes in Pregnancy," by George C. Thosteson, M.D., Assistant Clinical Professor of Medicine, Wayne State University, and Physician, Harper Hospital, Detroit; a movie, "The Diabetic Foot," by William E. Redfern, M.D., Associate Physician, Henry Ford Hospital; "Avoiding Dangers in the Management of the Juvenile Diabetic," by George H. Lowrey, M.D., Associate Professor of Pediatrics, University of Michigan, Ann Arbor; and "Recent Concepts in the Early Recognition of Diabetes Mellitus," by Stefan Fajans, M.D., Assistant Professor of Internal Medicine, University of Michigan.

A panel on "Diabetic Coma" was moderated by Gordon B. Myers, M.D., Professor of Medicine, Wayne State University, and Director of Education, Harper Hospital. Included in the panel discussion were Howard F. Root, M.D., Medical Director, Joslin Clinic, and Physician-in-Chief, New England Deaconess Hospital, Boston; Robert B. Leach, M.D., Assistant Professor of Medicine, Wayne State University; John B. Bryan, M.D., Associate Physician, Henry Ford Hospital; and Stefan Fajans, M.D.

Speaker at the evening session was Howard F. Root, M.D., whose subject was "The Relationship of Control of Diabetes to Vascular Complications."

NEWS NOTES

ADA MEMBERS PARTICIPATE IN POSTGRADUATE COURSES

Among the Officers of Instruction at Postgraduate Course No. 1 arranged by the American College of Physicians, Autumn-Winter, 1958-59, "The Physiological Basis of Internal Medicine," held October 13-17 at Duke University Medical Center, Durham, North Carolina, were Morton D. Bogdonoff, M.D. ("Calcium Metabolism" and "Advances in Pharmacology"); Frank L. Engel, M.D. ("Mechanisms of Hormone Action"); and William M. Nicholson, M.D. ("Radioactive Iodine").

"Selected Subjects in Internal Medicine" was the title of Course No. 2, held at the Mayo Clinic and Mayo Foundation November 3-7 at Rochester, Minnesota. Among the Directors of the Course was L. O. Underdahl, M.D., who was also Chairman of the November 3 morning session. Officers of Instruction included Charles M. Blackburn, M.D. ("Endocrine Considerations in Metastatic Breast Cancer"); Arthur R. Colwell, Sr., M.D., Chicago; Clifford F. Gastineau, M.D. ("Diabetic Acidosis: Some Principles of Treatment"); F. Raymond Keating, Jr., M.D. (moderator of "Symposium on Thyroiditis"); William M. McConahey, M.D. (participant in "Symposium on Thyroiditis"); Raymond V. Randall, M.D. (participant in "Symposium on Pituitary Disease"); Edward H. Rynearson, M.D. (moderator of "Symposium on Pituitary Disease"); Robert M. Salassa, M.D. ("Pituitary Lesions in Cushing's Syndrome" and participant in "Hypertension: Causes and Treatment"); Randall G. Sprague, M.D. (Chairman of November 3 afternoon session); and Dr. Underdahl ("Adenomatosis of the Parathyroids").

Titled "Gastro-Enterology," Course No. 3 of the American College of Physicians was held November 10-14 at the University of Michigan Medical School, Ann Arbor. Among the Officers of Instruction was Jerome W. Conn, M.D., who spoke on "Secondary Aldosteronism and Gastrointestinal Disease."

PERSONALS

JOHN E. HOWARD, M.D., Baltimore, participated in a symposium and panel discussion on adrenal steroids and their uses which was held September 25 at George Washington University. The symposium was sponsored by the Council on Drugs of the American Medical Association.

HERBERT POLLACK, M.D., New York City, spoke at the 41st Annual Meeting of The American Dietetic Association held in Philadelphia, October 21-24. The title of his paper was "Latest Research in Toxicity of Irradiated Foods."

NECROLOGY

CHARLES S. BYRON, Roslyn Heights, New York, born Sept. 15, 1898.

JACQUES R. FISCHL, New York City, born Jan. 20, 1914.

ADELINE MAY WESCOTT, Newburgh, New York, born July 18, 1870.